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SIR DIETRICH BRANDIS, 1824—1907.

By the death of Sir Dietrich Brandis, which occurred at Bonn, on May 28, 1907, a man of world-wide renown has been removed. He was born on April 1, 1824, at Bonn, being the son of Dr. Christian Brandis, Professor of Philosophy in the University of that place. As a boy, he followed his father to Greece, where he spent several years, and thus came into touch at an early age with the life and customs of the nearer East. On his return from Greece he was educated at the Universities of Copenhagen, Göttingen, and Bonn. In 1849 he established himself as "Privatdocent" on Botany at Bonn. While he thus started life as a Botanist, during his Botanical excursions his attention was soon turned to questions connected with the management of forests.

After the occupation of the Province of Pegu, in Burma, Lord Dalhousie offered Brandis the appointment of Superintendent of the Pegu teak forests, an offer which he accepted. He landed in India in 1856, and a year afterwards the rest of the Burma forests was placed under his charge. Brandis proceeded from Calcutta, where he had an interview with Lord Dalhousie, to Rangoon in a separate vessel from that which conveyed his herbarium and botanical library. The latter was wrecked in the Rangoon River, and Brandis thus lost his herbarium and books. He looked upon this almost as a direction to his future line of action. While he never quite abandoned botanical studies, he devoted for years his main energies to mastering the science and practice of Forestry. It was only thirteen years later that he resumed earnest botanical studies for the space of three or four years, and he then again returned to Forestry for a further period of nine years. After his retirement he occupied himself once more chiefly with botanical work.

From 1856 to 1862 Brandis worked indefatigably to bring the forests of Burma under systematic management. During these years a great conflict raged between the merchants of Burma and the Government, the former maintaining that the supply of teak timber from the forests was inexhaustible, and that, therefore, State interference was unnecessary. Brandis, supported by the Commissioner of Pegu, Major (afterwards Sir Arthur) Phayre, held different views. After a long continued struggle the forests were placed under systematic management, and they, with those of Upper Burma, are now the chief supply of teak timber to the world. If Brandis had done nothing else than save the Burma teak forests from destruction, he would have deserved well of the British Empire and the world in general.

In 1862, he was called to Simla at the suggestion, it is believed, of Dr. Cleghorn, one of the principal pioneers of forest conservancy in India, to advise the Government on forest matters in other parts of the country, and in 1864, he was appointed the first Inspector-General of Forests to the

Government of India. He then set to work to introduce systematic forest management on scientific lines throughout India. A regular department was created, and a forest law was passed which provided for the demarcation and management of the State forests. Brandis travelled from one end of the Bengal Presidency to the other, advising and organising the department. He also visited the Bombay Presidency twice, and he spent two years (1881—83) in Madras on special duty.

When he first started operations, he had to do with what staff he could lay his hands on, but he determined to obtain one fit to deal with the requirements of the case. There were already a few military officers in the Department, some of them medical men, and he began by inducing others to join. Some of these did excellent service, and they gave tone to the new Department. In 1866, while on sick leave in England, he obtained the sanction of the late Marquis of Salisbury, the Secretary of State for India, to educate young Englishmen in Continental forest schools, partly in France and partly in Germany. Under this system of training, which lasted until 1886, a number of distinguished Forest officers were supplied to India.

About the year 1881, a movement was set on foot to arrange for the education of Indian forest officers in Britain, and this led, in 1885, to the establishment of a School of Forestry in connection with the Royal Indian Engineering College at Coopers Hill. Brandis, who had then retired, was, of course, consulted about this move, and he did not approve of it, considering it to be premature. After some years, however, he agreed to superintend the practical training of the students on the Continent, from 1888 to 1896, when his functions ceased. On the closure of Coopers Hill, the forest branch was, in 1905, transferred to the University of Oxford. But Brandis went a step further. In 1878 he started a forest school at Dehra Dun for the training of natives of India, which now sends annually from forty to fifty trained executive officers into the Service. By these combined means a trained staff of 200 Englishmen and more than 1,000 Indians has been obtained which, assisted by about 10,000 subordinates, manages the Indian State forests, which comprise an area of 239,000 square miles, equal to one-fourth of the area of British India.

The results of Brandis' work in India are very remarkable. The supply of timber, firewood, and a variety of other produce to the teeming millions of India has been placed on a satisfactory footing; the productiveness of the forests is increasing year by year; the more important areas are protected against jungle fires, and the net revenue from the forests has risen from £40,000 in 1864 to £660,000 in 1904, although produce valued at a similar sum is given annually free of charge to the people of the country.

Brandis was equally interested in the indirect effects of forest vegetation. He started experimental stations at Dehra Dun and in Central India, where observations were made to test the effects of forests on temperature, humidity of the air and the soil, and the preservation of mountain slopes. His interest in the subject is testified by the fact that he was the first to compile a rainfall map of India, in 1871. It has been improved by subsequent observations, but as regards the main points it holds good to this day. The map served to show clearly the relation between the rainfall and forest vegetation in the several parts of India.

Brandis practically relinquished the post of Inspector-General of Forests in India in 1881, when he proceeded on special duty to Madras. He retired finally in 1883. On that occasion the Government of India acknowledged his services in the most complimentary terms, granting him not only a special pension, but a substantial gratuity in recognition of his specially meritorious services. He had been created a Companion of the Indian Empire in 1878, and he was promoted to a Knight Commandership in 1887 for his services in India, as soon as that grade was added to the Order.

Apart from British India proper, Brandis did all he could to encourage better forest management in the Native States. He had a considerable share in the development of systematic forest management in many of the Colonies by advising the Government of India to lend competent officers for service in various parts of the Empire, and by advice. After his retirement from India he continued to show uninterrupted interest in his great work by articles published in the 'Indian Forester,' and by letters of advice to his numerous friends in India.

As already mentioned, Brandis supervised the practical instruction on the Continent of the Coopers Hill Forest Students from 1888 to 1896. During that time, and afterwards, he also guided the studies of a number of young Americans, who have since established a great Forest Department in the United States dealing with State forests covering more than one hundred millions of acres. His influence in this respect has been so great that President Roosevelt, about a year ago, sent his picture to him with the following inscription: "To Sir Dietrich Brandis, in high appreciation of his services to forestry in the United States. From Theodore Roosevelt." Thus Brandis has left his mark upon every continent of the earth. As regards this country, his name will go down to posterity as the founder of systematic forest management in the British Empire.

Brandis was not only a great administrator, but also a scientific man of a high order. During his career in India he wrote an endless number of reports and papers, and in 1872—74 he interrupted his forest work by writing the "Forest Flora of North-west and Central India," a work so highly thought of that he was elected a Fellow of the Royal Society in 1875.

The last eight years of his life were devoted by him to the writing of a general Indian forest flora, which he published in 1906 under the title of "Indian Trees," a monumental work which is likely to be the standard book of reference on the subject for another generation.

Of other publications, the following may be mentioned:—

1. "Vegetation and Country from Narkanda to Pangi." Simla, 1879.
2. "The Ringal of the North-western Himalaya." 1885.
3. "Die Nadelhölzer Indiens." 1886.

4. "Wall Pictures to Illustrate the Minute Structure of Plants." Simla, 1886 and 1887.

5. "Three Families of Plants in Engler and Prantl's 'Die natürlichen Pflanzenfamilien.'" 1894.

6. "Dipterocarpaceæ." Brandis and E. Gilg, 1894.

7. "Geographische Verbreitung der Dipterocarpaceen." 1897.

8. "Ueber die geographische Verbreitung der Bambusen in Ostindien." 1896 and 1897.

9. "Biological Notes on Indian Bamboos." 1899.

10. "Anbau der grossen Bambusen in Deutsch Ostafrika." 1899.

11. "On Some Martaban Bamboos." 1906.

12. "Remarks on the Structure of Bamboo Leaves," in the 'Transactions of the Linnean Society.' 1906.

13. "The Spruce of Sikkim and the Chumbi Valley." 1906.

Brandis was a Fellow of the Royal Society, the Linnean Society, and the Royal Geographical Society, and LL.D. of the University of Edinburgh; an Honorary Member of the Royal Scottish Arboricultural Society, the Society of American Foresters, and of the Pharmaceutical Society of Great Britain.

He had scarcely completed his great work, "Indian Trees," when he fell ill. After a painful illness of several months' duration he died at the age of 83 years, thus bringing to a close a most remarkable life filled with work from beginning to end, which only his iron constitution enabled him to achieve. He never spared himself, and he was always a warm friend of those associated with him in his work. For the natives of India he was full of sympathy, and he did all he could to advance their education and fit them to partake in the administration of the country, and more particularly of the Forest Department. He married, in 1854, Rachel Shepherd, a daughter of Dr. Marshman, of Bengal. She accompanied him to India, where she died in 1863. In 1867 he married Katherine, daughter of Dr. Rudolph Hasse, of Bonn, who survives him. He left three sons and one daughter.

W. S.

SIR WILLIAM HENRY BROADBENT, BART., K.C.V.O., 1835—1907.

SIR WILLIAM HENRY BROADBENT, born in 1835, was the son of John Broadbent, of Longwood Edge, Huddersfield. He married, in 1863, Eliza, daughter of Mr. John Harpin, of Birks House, Holmfirth, Yorkshire, by whom he had two sons and three daughters. His two sons, Dr. John Francis Harpin Broadbent, who succeeds his father in the baronetcy, and Dr. Walter Broadbent, are both members of the medical profession.

Broadbent had a distinguished career as a student of medicine and afterwards as a great physician. He took a distinguished place in the medical world not only as a practitioner, but as an original investigator of difficult medical questions, and of physiological subjects bearing on the science and practice of medicine. He had the originality of a man who thought for himself; originality shown in finding out new things and not merely in developing a little further the discoveries of others. His professional success was great and well deserved.

In 1892 he was appointed physician-in-ordinary to H.R.H. the Prince of Wales, now King Edward VII. Next year a baronetcy was given him, and in 1901 he was made a Knight Commander of the Victorian Order. Nor were honours of this kind limited to those conferred in this country; he was invested with the Grand Cross and Insignia of the Legion of Honour. In 1898 he was appointed physician-extraordinary to Queen Victoria, and, later, physician-in-ordinary to King Edward.

Besides these honours he received numerous academic distinctions, including the honorary LL.D. of the Universities of Edinburgh, St. Andrews, and Toronto, and the honorary D.Sc. of the University of Leeds. He was elected a Fellow of the Royal Society in the year 1897.

That Broadbent was a man of high intellectual power and of great nobility of character is well known to his friends, and is evidenced by his career and also by the nature of the original medical and physiological work he did. His contributions to medical literature cover a wide and varied field. He was deeply interested in the study of the problems of diseases of the nervous system. One of his earliest publications dealing with this subject was an important paper entitled "The Sensori-Motor Ganglia and Association of Nerve Nuclei," in which he enunciated his memorable "hypothesis" explaining the immunity from paralysis of bilaterally associated muscles in hemiplegia. In 1869 he published a paper on "The Structure of the Cerebral Hemisphere," in which he gave a description of the course of the various groups of nerve fibres in the cerebral hemisphere, based on a series of careful dissections which he had been carrying out for some years. In a lecture on "The Theory of Construction of the Nervous System," delivered at Wakefield in 1876, he referred to these dissections, and gave a lucid exposition of his views on the mechanism of speech and thought, and the problems of aphasia, a subject in which his interest was maintained throughout his life. To this his papers on "A Case of Amnesia," on "A Particular Form of Amnesia," "Loss of Nouns," read before the Royal Medical and Chirurgical Society in 1878 and 1884 respectively, and an article in the 'British Medical Journal' as late as June 15 this year, on "Some Affections of Speech," bear witness. Indeed, at the time of his death he was engaged in writing a treatise on "Aphasia." Amongst other publications dealing with diseases of the nervous system, were "Remarks on the Pathology of Chorea," published in the 'British Medical Journal' in 1869, the Lettsomian Lecture on "Syphilitic Affections of the Nervous System," delivered before the Medical Society of London in 1874, and papers on "Ingravescent Apoplexy" and on "Alcoholic Spinal Paralysis," read before the Royal Medical and Chirurgical Society in 1874 and 1884. In 1866, the year in which he published his "Hypothesis," he contributed an article on "Prognosis in Heart Disease" to the 'British Medical Journal.' In 1884, before the Harveian Society of London, he gave the Harveian Lectures on "Prognosis in Valvular Disease of the Heart." In 1887, at the Royal College of Physicians, he delivered the Croonian Lectures on the "Pulse," and in 1891 the Lumleian Lectures on "Structural Diseases of the Heart from the Point of View of Prognosis." These lectures constituted the foundation of a work on "Heart Disease," published in 1896, in the preparation of which he was assisted by his son, Dr. John Broadbent, and of which a revised and enlarged edition appeared in 1906.

His early interest in the scientific application of therapeutics was shown by a paper entitled, "An Attempt to Apply Chemical Principles in Explanation of the Action of Remedies and Poisons," published in 1869; and the line of thought in this was followed out in later years in an address on "The Remote Effects of Remedies," read at the annual meeting of the British Medical Association in 1886, and in his Presidential Address delivered before the Clinical Society on "The Relation of Pathology and Therapeutics to Clinical Medicine." Amongst other notable contributions to the literature on therapeutics is the Cavendish Lecture on "Some Points in the Treatment of Typhoid Fever," which he delivered before the West London Medico-Chirurgical Society in 1894.

Sir Thomas Barlow has observed that, in a remarkable paper submitted several years ago to the Royal Medical and Chirurgical Society, but not published in its 'Transactions,' Broadbent anticipated the development of pharmacology on the lines of chemical affinities of the elements.

Broadbent has left a record of splendid work done in the advancement of neurology. The value of what he did towards the elucidation of different problems presented by cases of aphasia is universally acknowledged. This is not the place to give details of any of his researches. I shall limit my remarks to the wide bearings of a great principle he established, to what is known as "Broadbent's hypothesis." This principle has brought method into the analysis of complex symptomatologies of some very different nervous maladies. More than forty years ago ('British and Foreign Med. Chir. Review,' April, 1866), he published an article on "the Bilateral Association of Nerve Nuclei to the Higher Centres." These words, his latest but not his best, deliverance on the subject, are from a lecture he delivered before the Neurological Society, published in 'Brain,' No. 103, p. 347, 1903. In that lecture he remarked that: "The principle is that when muscles on the two sides of the body always act together, their nuclei, situate in opposite sides of the cord, are so closely associated by commissural fibres as to be practically one nucleus." Whatever modifications and corrections of details have been required, what is essential in the principle which the hypothesis embodies has not been invalidated. Thus, in a case of ordinary hemiplegia—say right—owing to disease of the left half of the brain there is loss of power of the right limbs, that is, of those parts of the body which are most nearly unilateral, and, as we may say, "most voluntary," in their actions; but there is no, or very little, disability of the intercostal muscles of either side of the chest. Nevertheless, there is loss of many *movements* of the intercostal *muscles* of *both sides*, that is to say, there is paralysis *in the sense of loss of movements* of the intercostal muscles of both sides, but without disability of these muscles. The seeming paradox in this statement disappears when we reflect that, as Broadbent told us long ago, in such a case the intercostal muscles of both sides of the chest remain represented in another set of movements in, and are still empowered by, the undamaged right half of the brain; there is almost complete compensation by the right half for the effects of the destruction lesion in the left half. The truth of this hypothesis is demonstrated in two ways. In some cases of what may here be called Rolandic epilepsy (it is sometimes called "cortical epilepsy") there is from a local lesion of the motor region of the cortex cerebri of one half—say, left—of the brain occasionally convulsion of the limbs of the right side of the body and of both sides of the chest. In the facts of hemiplegia contrasted with those of the case of Rolandic epilepsy there is a striking verification of Broadbent's hypothesis. From a destruction lesion of part of one half of the brain there is no, or very little, obvious disability of the intercostal muscles of either side, whilst from a discharge lesion of a part of the cortex (a part belonging to the same anatomico-physiological system as that part which is the seat of a destruction lesion in hemiplegia) there is great spasm of the intercostal muscles of both sides. Another, a third, confirmation of the hypothesis is given by so-called pseudo-bulbar paralysis in cases of this malady there is a double cerebral destruction lesion, causing great disability of bilaterally acting muscles of a certain region of the body—of both sides of the tongue, lips, and palate. A destruction lesion of the left half of the brain only causes very slight, almost no, disability of the bilaterally acting parts mentioned, compensation being for the effects of that one-sided lesion practically complete. But when that compensation is lost from a lesion of the right half also, there is very great disability of the bilaterally acting parts mentioned. Speaking more at large, it follows from Broadbent's hypothesis that "double hemiplegia" is more than the double of hemiplegia.

So much for three applications of the hypothesis. There is another one of great importance to which allusion may be made. The hypothesis "leads ('Brain,' *op. cit.*) to the conclusion that words are represented in the right as well as in the left hemisphere." (Broadbent, 'Brain,' *loc. cit.*)

From what has here been said, it will be seen how fundamental and of what wide application Broadbent's hypothesis is. This basic contribution to neurology has lasted forty years, and is still not only valuable for the explanation of certain neural symptomatologies, but is also fruitful in its indications for further research in medical neurology. Moreover, the writer thinks that it and deductions or inferences from it, will be found of great value in the study of still larger problems than those here dealt with, such as investigations into the physiology of the organism, when that physiology is considered as especially corresponding to psychology, both to the psychology of the sane and of the insane. J. H. J.

NOTE.—Much of this Obituary is taken from an article, part of which was contributed by the author, in the 'British Medical Journal,' July 20, 1907.

WILLIAM TENNANT GAIRDNER, 1824—1907.

WILLIAM TENNANT GAIRDNER was born in Edinburgh on November 8, 1824 and he died in the same city on June 28, 1907. Descended from an Ayrshire stock, he was the son of Dr. John Gairdner, who was for many years a well known medical practitioner in Edinburgh. His mother was Susanna Tennant, a granddaughter of the Rev. Dr. Dalrymple, of Ayr, the "Dalrymple mild" of Robert Burns. Gairdner was educated at the University of Edinburgh, where he graduated in 1845. The teachers who appear to have influenced him most were William Alison, the physiologist, and Robert Christison, the most distinguished pharmacologist of his day. After a short sojourn in Italy, in the company of Lord and Lady Beverley (afterwards the Duke and Duchess of Northumberland), he returned to Edinburgh, was appointed one of the resident assistants in the Royal Infirmary, pathologist to the Infirmary in 1848, obtained wards in 1853, and became an extra-mural lecturer on Practice of Medicine in the same year. He was elected to the chair of Practice of Medicine in the University of Glasgow in 1862, and this office he held till his retirement in 1902. In 1863, Gairdner was appointed the first "Medical Officer of Health" for Glasgow, a position he held for nine years. He was President of the British Medical Association in 1888, became a Fellow of the Royal Society in 1893, and, in 1898, Queen Victoria honoured him by creating him a Knight Commander of the Bath. He also represented his University on the General Medical Council from 1893 to 1902. He was President of the College of Physicians of Edinburgh during the years 1893–5. In 1870 he married Miss Helen Bridget Wright, of Norwich, who, with several sons and daughters, survives her husband.

In his long and varied career Gairdner engaged in many kinds of work, and the record of his life must, therefore, present various aspects, according as we view him as pathologist, clinical physician, sanitarian, and man of letters. In the early Edinburgh days, while Pathologist to the Royal Infirmary, he devoted special attention to the pathology of the kidney, and, in particular, gave an early description of the waxy kidney. About 1850, he investigated the pathological changes in bronchitis, and more particularly diseases of the lung associated with bronchial obstruction. He enunciated a theory of emphysema, accounting for the changes in the air cells of the lung in that condition by the force of the inspiratory instead of the expiratory act. This theory has not been generally adopted. But Gairdner's contributions to pathology were not so much in the form of specific investigations as in the general trend of his clinical work. Every case that came before him was subjected to the most rigid scrutiny, not merely in its clinical aspects, but in the verification and correction of these by the facts of the *post-mortem* theatre. In the case books of the Royal Infirmary of

Edinburgh and of the Western Infirmary of Glasgow, there are painstaking records of the most valuable description, monuments of industry, insight, and method.

As a clinical physician he belonged to the first rank of workers. In the old days this part of investigation, especially as regards the teaching of students, was carried on in a very loose fashion. The physician often passed from bed to bed in the wards, followed by a crowd of students, who were left to gather wisdom and knowledge as they could from the oracular utterances of the wise man. There was no systematic investigation of cases nor any attempt at methodical teaching of the student. The first great improvements were no doubt initiated by John Hughes Bennett, who, fresh from the cliniques of the Parisian hospitals, was the first in this country to teach the student at the bed side, by causing him to observe the facts of the case, and to discuss, with the teacher, all its features. Gairdner readily took up the same course of procedure. More thorough and philosophical than Bennett, he went more deeply into the case, and worked out what may be termed its natural history. He disliked clinical pictures. He disliked the practice of drawing up an imaginary schedule for each disease with a space for each sign and symptom, which had to be filled in whether it happened to be in the case under discussion or not. His method was rather to study each individual case and to closely scrutinise each symptom, to note those belonging to the typical form of the disease that were absent in this particular case, and to draw inferences with care and precision. It was the scientific method that impressed the student and trained him to be an observer. Gairdner always attracted to him the better type of men, and many of his pupils became, in their turn, able pathologists and physicians. In practical therapeutics he also did valuable work. He was one of the first to check the over-stimulating mode of treatment in fevers and pneumonia, and when he did use medicinal substances they were employed in a simple form. He never wrote a "grape and canister" prescription containing half a dozen ingredients. He had no great faith in drugs, nor in specially vaunted remedies, or modes of treatment. There must be, in his view, a good reason for the employment of a particular remedy and he was not guided by empirical considerations, except to a very limited extent. As a consultant, therefore, he was often somewhat disappointing, as he was more interested apparently in the clinical history of the case and in its pathology than in the treatment. He had a strong view that in many cases natural processes would remove the disease, while, in others, so-called remedies were of little avail.

One of Gairdner's chief claims to distinction is the splendid work he did as the first medical officer of health of Glasgow. During the first half of the nineteenth century, and closely connected with the industrial revolution that followed the introduction of machinery and the factory system, many large towns rapidly increased in population and unsanitary conditions of life were met with everywhere. In no city was this more conspicuous than in

Glasgow. In 1801 the population was 83,805; in 1811, it had risen to 110,400; in 1821, to 147,043; in 1831, to 202,426; in 1841, to 280,692; and in 1851, to 347,001. In 1818, there was a severe epidemic of typhus; in 1832, this was repeated, with, in addition, an epidemic of cholera, and a death-rate of 46 per 1000. In 1837, there was another typhus epidemic; 1843 brought an epidemic of relapsing fever; 1847 had a typhus epidemic with a death-rate of 56 per 1000; 1848–9 was visited by a second cholera epidemic, in which 3772 deaths occurred; 1851–2 showed more typhus; and 1853–4 had a third cholera epidemic with 3885 deaths, and a death-rate of 42 per 1000. It is now difficult to realise exactly what those figures mean, and yet this must be attempted if we wish to understand the real state of matters. Thus, in 1837, the population of Glasgow was 253,000, the death rate was 41 per 1000, and the number of deaths from "fever" was 2180, or 8·6 per 1000. At least 21,800 persons suffered from the disease during that year. In 1847, the number of persons affected by fever must have been about 45,000. If we wish to contrast this with the state of matters in Glasgow at the beginning of the present century, we may take 1901, said to be the worst epidemic year of recent years.* In 1901, the population of Glasgow was 761,712; the general death-rate was 20·6; the number of deaths from fever was 220; the number of cases of fever notifiable, 1385; and the number of deaths from all infectious diseases 3416, or 4·4 per 1000 living. The total number of cases of infectious diseases registered during the year was 21,145, or less than the number believed to have suffered from "typhus" fever alone in 1837.

The cause of the terrible state of matters that prevailed up to the passing of an important municipal act in 1862 was undoubtedly due to overcrowding in insanitary dwellings, to the entire absence in the poorer parts of the city of even the most obvious sanitary appliances, and to the want of hospitals for the segregation of the sick during an epidemic. In January of 1862, Gairdner was chosen to be the first medical officer of health, and five district surgeons of police were appointed his assistants. At the same time, a "special non-medical inspector" represented the entire sanitary staff. The first sanitary office was a room measuring fifteen feet by ten feet. These rudiments of a sanitary department soon developed. Disinfecting and washing houses were established, nuisances were removed, careful inspection was made of specially insanitary districts, committees were formed for special purposes, intra-mural burial grounds were closed, and a hospital for fever was founded at Belvidere, and became in course of time one of the finest fever hospitals in the country. Year by year the sanitary machinery was improved and in particular the danger of overcrowding was combatted by means of "ticketing" houses, that is, specifying on the door of each room the number of inhabitants who were permitted to dwell in it. During this period Gairdner wrote many important papers, on sanitary questions, on hospital management, on the training of nurses, on dietetics both as regards the healthy and the sick, and on kindred subjects. In 1871, he resigned the office of medical officer of health into the able hands of James B. Russell, one of his own most distinguished pupils, who carried on for many years the work of the Sanitary Department in Glasgow, which is now one of the most complete in the world. Its initiation owed much to Gairdner and he had the satisfaction of watching its progress.

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* These figures have been obtained from a paper from the late Dr. J. B. Russell, entitled, 'The Evolution of Sanitary Administration in Glasgow.' The facts regarding 1901 are given by the present medical officer of health of Glasgow, Dr. A. K. Chalmers, in a footnote to Dr. Russell's paper, as it appears in a volume entitled, 'Public Health Administration in Glasgow,' a memorial volume of the writings of Dr. J. B. Russell, who succeeded Gairdner as medical officer of health. See pp. 1, 4, etc.

Gairdner in many directions showed marked literary gifts. He was a good classical scholar, of a type not common now-a-days in the medical profession, and he read Virgil and Horace and the New Testament in Greek, as one of his almost daily pastimes. Now and again he published, in addition to works bearing specially on medicine, isolated lectures and essays, such as 'The Physician as Naturalist,' a well-known volume which is a key to his character. Always animated by a thoroughly scientific spirit, he took a wide view of the functions of a physician, as one whose duty it was to investigate the natural phenomena of disease, and uphold the dignity of his calling. In his writings, as in his conversation, one always felt conscious of his lofty ideals and of his transparent sincerity and honesty of purpose. It would not be right not to advert to another marked characteristic. He was a man of sincere piety who ever lived in his daily life under the shadow of the Unseen. Broad and catholic in his religious views, there was always present the spirit of reverence, a generous appreciation of the views and claims of others, a desire to view every question fairly and impersonally, and a high ideal of the nobility of life.

J. G. McK.

ROBERT WARINGTON, 1838—1907.

THE name of Robert Warington will ever be associated with one of the most important advances in the agricultural chemistry of the latter half of the nineteenth century, though his classical work on nitrification, which may be regarded as his life-work, bears but a small proportion to the total of that accomplished by him. He, no doubt, owed his chemical proclivities to his father—a Robert Warington also—who was a prominent figure amongst the chemists of earlier days. The elder Warington was one of the first chemical assistants at University College, and was subsequently appointed chemical operator to the Society of Apothecaries. He, also, was a Fellow of the Royal Society, and published several papers on chemical subjects; yet chemistry is more indebted to him for the part which he took in founding the Chemical Society than for the extent of his own original work. It was through his zeal and powers of organisation that this Society was founded in 1841, and the work which he did for it as its secretary during the subsequent ten years helped in no small measure to launch it on its prosperous career.

Robert, his eldest son, was born on August 22, 1838, in the parish of Spitalfields. His mother was a daughter of George Jackson, M.R.C.S., to whom science is indebted for several improvements in microscopes which have not yet been superseded, as well as for the invaluable ruled glass micrometer. The original dividing machine made by him for ruling the lines was still being used by a well known optician in 1899, and is probably in use at the present time.

Very early in young Warington's life his parents took up their residence at the Apothecaries' Hall, and it was here, in the uncongenial atmosphere of the city, that he spent his childhood and youth. His constitution was naturally feeble, and a life in the heart of London, with but little exercise, and no companions of his own age to assort with, did not tend to strengthen it. All through life he had to contend with a lack of bodily vigour, which rendered his work doubly laborious to him. For his education he seems to have been chiefly indebted to his parents. While still quite young, he studied chemistry in his father's laboratory, and had the advantage of attending lectures by Faraday, Brande, and Hofmann.

In consequence of the unsatisfactory state of young Warington's health, his father sought to get him some employment in the country, and, with that object in view, applied to Mr. Lawes, with whom he was acquainted, and for whom he had done some professional work. The outcome of this was that in January, 1859, the youth went to work in the Rothamsted Laboratory as Lawes' unpaid assistant. Here he remained for one year, devoting all his time to ash analyses, of which he had had no previous experience, and examining various methods for obtaining the most satisfactory results. Dr. Pugh and Mr. F. R. Segeleke were also working in the laboratory at that time, and they gave Warington valuable assistance in his work. Of the two series of analyses eventually completed, the first comprised those of the ashes of grass grown under different manurial treatment, the results of which were published in Lawes' and Gilbert's "Report of Experiments with Different Manures on Permanent Meadow Land,"* the second series was that of the ash of grain from Broadbalk Field. These latter analyses were never published, their place having been taken by more complete work on the same subject by Richter.

Although Warington left the Rothamsted Laboratory in January, 1860, his interest in the work there never ceased, and, until he resumed his connection with Lawes a few years later, he devoted much of his time to studying the Rothamsted results, and was a frequent visitor to the laboratory.

His health having been somewhat re-established by his year's residence in the country, he returned to town, and continued to reside with his parents till 1862, spending his days at South Kensington, where he worked, under Dr. Frankland, as research assistant. But at the end of this period, a further breakdown in health forced him again to seek a country life, and he betook himself to the Royal Agricultural College at Cirencester. Here he remained for four and a-half years, the first nine months of which were spent in doing analyses for Dr. A. Voelcker, and the remainder of the time in fulfilling the duties of teaching assistant under Professor Church.

It was during his residence at Cirencester that Warington published the first papers on scientific subjects which appear under his name. These were printed in the 'Journal of the Chemical Society.' The earliest of them (1863) dealt with the quantitive determination of phosphoric acid. This was followed by two other short communications on kindred subjects, which preceded and prepared the way for his first work of importance—an investigation into the part played by ferric oxide and alumina in decomposing soluble phosphates and other salts, and retaining them in the soil. The results of this investigation are embodied in a series of four papers read before the Chemical Society, and are typical examples of the careful work and close reasoning which characterised all Warington's researches. That ferric oxide acted as a fixing agent for soluble substances applied to a soil, was already known, but the action was attributed to an indefinable physical attraction, which explained nothing. Warington proved, first by experiments with pure ferric oxide, and then with ordinary soil, that the action in the case of calcium phosphate was simply one of chemical decomposition, resulting in the formation of ferric phosphate, whilst, in the case of other salts, such as carbonates, sulphates, nitrates, etc., the chemical character of the action was indicated by the fact that the iron did not retain the salt as a whole, but partially decomposed it, retaining the basic portion in excess over the acid portion.

Warington did not allow his work at Cirencester to sever his connection with Rothamsted, and he offered to analyse three of the most important of the animal ashes which had been prepared there, on the condition that he might make use of the results thus obtained. He consequently received mixed ashes representing the whole bodies of a fat ox, a fat sheep, and a fat pig, and an abstract of the analysis made by him appeared in an article which he wrote for the second supplement to "Watts' Dictionary of Chemistry." The analysis, together with others by Richter, were also published by Lawes and Gilbert in the 'Phil. Trans.,' 1883.

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* 'Journ. Roy. Ag. Soc.,' vol. 20, 1859, p. 407.

In 1864 Warington commenced lecturing to the students at Cirencester on the Rothamsted experiments, and went systematically through all the work which had already been published, together with many additions of as yet unpublished results which had been communicated to him by Lawes and Gilbert. A desire was expressed at Cirencester that these lectures should be published, and negotiations to that end were consequently opened with Lawes and Gilbert. The outcome of these was that Warington was to write a book on the Rothamsted investigations, Lawes guaranteeing him from pecuniary loss, but offering no remuneration. Lawes also reserved to himself the right to supply a preface to the book, on the ground that there would be previously unpublished matter incorporated therein. The writing of this book involved a large amount of labour, especially as, in studying the effect of manures in different seasons, Warington was led to recognise the almost paramount influence of the rainfall on the results, and its action in washing the nitrates out of the soil, an action up to that time unrecognised. For the purpose of examining this action more closely, he compared the results from the plots at Rothamsted with the temperatures and rainfalls supplied to him by Glaisher; at the same time he applied to Gilbert to furnish him with unpublished data respecting the Rothamsted hay crops. Gilbert, however, objected to what now appeared to him in the light of a publication of Rothamsted results by others than Lawes and himself. Discussions ensued, the upshot of which was that the book remained in manuscript, and the seeds of an unfortunate dissension between Gilbert and Warington were sown. Some 120 pages of this book were written (and are still in existence), but Warington declined the pecuniary compensation which Lawes offered to him for his labour.

Leaving Cirencester in June, 1867, he became chemist to Lawes' manure and tartaric and citric acid factories at Millwall, where he remained till 1876. During these years he generally had a long conversation every week with Lawes on those problems in agricultural chemistry which happened to be under investigation at the time, and which were evidently more congenial subjects of discussion to both of them than the problems arising in the factory. Even these, however, were by no means lacking in interest, and at the conclusion of his engagement at Millwall in 1874, Warington remained in the laboratory there for two years longer, working on citric and tartaric acids, and ultimately publishing his results in a paper of 70 pages in the 'Journal of the Chemical Society.' This paper was published with Lawes' approval, and it is noteworthy for the opinion expressed therein, that "the large amount of information acquired in the laboratories of our great manufacturing concerns might well be published without any injury to the individual manufacturer." Eighteen years later, when Warington had for a second time gone to work in Lawes' tartaric and citric acid factory, he published another paper dealing with these acids, and with the detection of the presence of lead in them. With this solitary exception, all Warington's subsequent work was on agricultural chemistry, and all of it was done in the Rothamsted laboratory.

While still at Millwall, he had been writing a good deal on agricultural subjects—several articles for "Watts' Dictionary" and for the Agricultural and Horticultural Co-operation Association—and he had, moreover, as already mentioned, been in continual consultation with Lawes as to the Rothamsted results; he was naturally, therefore, prepared to receive Lawes' suggestion that he should go and work in the Rothamsted laboratory. The terms were all settled, and had readily been assented to by Warington; for, although they had involved a reduction of salary to two-thirds of that which he had been receiving at Millwall, he obtained a certain amount of freedom by way of compensation. He was to be at liberty to publish his own work in his own name, provided that it made its appearance as Rothamsted work; but in cases where the work dealt with subjects which had already occupied the Rothamsted investigators, it was to be published in the joint names of Lawes, Gilbert and Warington. This arrangement, however, owing to some unforeseen difficulties, was not carried out; and it was not till after a delay of two years that Warington went to Rothamsted (in 1876), under an agreement for a year only, to work simply as Lawes' private assistant. The engagement was subsequently extended, and all his results were published, either in his own name or in the names of Lawes, Gilbert and Warington.

Before removing to Harpenden, he went to work at the laboratory at South Kensington in order to learn water and gas analysis under Frankland's assistant, some of the Rothamsted soils being sent to him for practising determinations of nitrogen. While there he devised a method of extracting soils by the vacuum pump, which method has since been largely used at Rothamsted. In the autumn of the same year (1876) he made a short tour among the German experimental stations, and then took up his residence for good at Harpenden.

The construction of a gas analysis apparatus (under Frankland's direction) for the Rothamsted laboratory occupied a considerable time, and, pending its completion, Warington made a study of the indigo method of determining nitric acid. This method, as generally used, he found to be full of sources of error. The principal of these he succeeded in correcting, and, with the method of determination, thus rendered trustworthy, he proceeded to determine regularly the nitrates in the drainage-water from the various wheat plots in Broadbalk field. The chlorides were determined at the same time. No such systematic work had been previously done; whilst the methods of sampling, which had been adopted when any analysis had been made, had been faulty. Warington now altered these methods, so that the samples analysed should faithfully represent the average composition of the drainage-waters.

Having examined the indigo method for determining nitric acid, he next examined the Crum-Frankland method by agitation with mercury; and, subsequently, the method of Schlœsing, modified, however, in such a way that the nitric oxide produced was determined by gas analysis. The exhaustive examination of these methods of analysis are described in a series of papers published in his own name in the 'Journal of the Chemical Society' and elsewhere, extending down to 1882. The modified Schlœsing method was the one which he finally adopted, and with it he began a long series of determinations of nitrates in soils, and in mangels, swedes, and potatoes.

Having satisfied himself as to the methods of nitrogen determination, he next turned his attention to those for the estimation of carbon, and having examined the permanganate and the bichromate methods, and found them wanting, he finally adopted the combustion method, which proved to be thoroughly satisfactory, provided that carbonates were entirely removed by prolonged treatment with sulphurous acid. In this work he was assisted by Mr. W. A. Peake, and the results were brought before the Chemical Society in the names of Warington and Peake.

Warington's results from the examination of the rain and drainage water, together with results previously obtained at Rothamsted, formed the subject of a very long report published in the names of the three investigators in the 'Journal of the Royal Agricultural Society' for 1882. The subject, however, continued to occupy Warington's attention long after this date, and we find a report on the subject in the three joint names in 1883, and papers by Warington alone in 1889 and 1887. The last-mentioned paper is an important contribution* to the study of well waters, and deals with the wells in the chalk formation on which Harpenden is situated. In later years (1904) Warington was enabled to give these results a practical bearing on the supposed contamination of the Harpenden water supply, and he saved the community, at any rate, for a time, from adopting an expensive, and, apparently, quite unnecessary system of sewerage.

So far, Warington's work, as here described, consisted largely of examining and perfecting methods of analysis for use in agricultural research. For this work the precision of his nature, and the carefulness of his manipulation pre-eminently fitted him, and most of the methods of analysis which he elaborated have been accepted as standard methods, which promise to remain in use for many years to come. The remainder of his work, however, is that by which he made his name, and, if a strictly chronological sequence of events had been followed, it should have been mentioned earlier in this notice, for it was in 1877 that he began to study nitrification, and this subject occupied the foremost place in his mind till 1891, when his opportunities for pursuing the subject ceased. During this period he published about ten papers on the subject, all in his own name, the principal of which were four communications to the Chemical Society, bearing the title "On Nitrification," Parts I to IV.

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* 'Journ. Chem. Soc.,' pp. 500—552.

That the natural conversion of ammonia into nitric acid was the work of an organism, had been suggested by A. Müller as early as 1873, but it had been reserved for Schlœsing and Müntz to establish definitely that this was the case. In 1877 they showed that, when sewage was allowed to percolate through a column of sand and limestone, the nitrification which occurred during its passage could be prevented by the presence of a sterilising agent such as chloroform vapour, and, after such sterilisation, the activity of the sand could be resuscitated by inoculating it with a few particles of vegetable mould. Questions affecting the problems connected with nitrogen in the soil had naturally been amongst those to which the Rothamsted investigators had, from the first, devoted themselves, and, consequently, they at once set to work to examine such an important observation as that of Schlœsing and Müntz. A complete verification of it was obtained by Warington, operating with garden soil only, and a solution of ammonium chloride, instead of sewage; and he was enabled to add the additional information, that nitrification occurred only in the dark. This paper appeared within a year of that of Schlœsing and Müntz. Two and a-half years later he published a second paper which added considerably to the facts already established. He showed that the nitrifying organism, besides requiring darkness in order to do its work, must also be supplied with food for its growth—potash, lime and phosphorus—and moreover, that all liberation of free acid must be prevented by the presence of some salifiable base, such as calcium carbonate. He found, also, that after the introduction of a small quantity of active soil or solution into a liquid capable of nitrification, no action occurred till a certain time had elapsed, this period of incubation being probably due to the organisms having to multiply to a certain extent before they become sufficiently numerous to produce recognisable results. An increase of temperature was found to favour the action up to a certain point, and it was shown that various vegetable moulds and known bacteria were not the organisms to which nitrification could be attributed. Many difficulties, however, still remained to be cleared up, notably the want of uniformity of the action, which resulted in the production of nitrates in some instances, and nitrites in others. We now know that the process is performed by two quite distinct organisms, and that their nutrition is, in some respects, wholly different from that of any other organism hitherto studied; but until this knowledge was gained, work on the subject was singularly difficult, and the results were very perplexing.

Warington's third paper on nitrification added considerably to our knowledge of the circumstances attending the action, and established the fact that the organisms are almost entirely confined to the first nine inches of ordinary soil. The distribution of the organism in the soil was dealt with still more exhaustively in a subsequent communication in 1887.

The prize coveted by the workers on this subject was, however, the isolation of one organism itself; and to prepare himself for this task Warington went to London for a time, in 1886, to learn bacteriology under Dr. Klein at the Brown Institution. From Dr. Klein he obtained a large number of pure cultures of various bacteria, and all these, as well as others obtained from his own experiments with soils, he examined as to their behaviour towards ammonia and nitrates, and also as to their mode of growth on skim-milk. The results were brought before the Chemical Society, and proved that none of the bacteria, except the nitrifying organism itself, possessed any appreciable power of nitrification. The majority of the organisms examined, however, were active denitrifiers. Denitrification—whereby nitrates are converted into nitrites, oxides of nitrogen, or even nitrogen gas—was, at this time, a well recognised work of micro-organisms, but was one which, naturally, enhanced to a considerable extent the difficulties met in elucidating the reverse phenomenon of nitrification. Warington's work added a good deal to our knowledge of the subject, and showed that denitrification is a property actively exhibited by a large number, but by no means by all, micro-organisms, and that in a soil it becomes complete, before the nitrifying organisms begin their task of reversing the reaction. An excellent account of the denitrification of farmyard manure was subsequently written for the 'Journ. Roy. Ag. Soc.,' 1897, vol. 8, Part IV.

Warington's work on nitrification was amply sufficient to establish the fact that the oxidation of ammonia in the soil was the work of an organism, but that organism seems to have been isolated first by Schlœsing and Müntz in 1879, though the method which they adopted left, at the time, considerable doubt as to its real identity. But even the isolation of this organism did not solve the whole problem: there was still the independent formation of nitrites and nitrates to be accounted for; and it was here that Warington's work was most conducive to a solution of the difficulties, for he succeeded in proving that one organism alone could not be held accountable for the various phenomena observed, and that two different organisms must be concerned in the process of nitrification. His success all lay in the chemical aspects of the subject. He was the first to obtain (1879) liquid cultures which converted ammonia into a nitrite, and preserved this power in all sub-cultures, but which was incapable of producing any nitrate; and shortly afterwards (1881) he obtained cultures which were able to convert nitrites into nitrates, but were unable to oxidise ammonia. This was a practical separation of two distinct organisms, but at the time Warington did not grasp the true meaning of his results, and he associated the change from nitrites into nitrates with a white growth which appeared floating in the liquid, but which really had nothing to do with it.

In 1890, after the work of others had resulted in the isolation of the nitrous organism (that which converted ammonia into nitrites), Warington returned to the subject, and found that the white surface organism could not be held accountable for the conversion of the nitrites into nitrates. He eventually succeeded in isolating the organism which really produces this change, and obtained a nearly pure culture of the nitric organism. At the same time he showed that organic carbon is not necessary for the growth of these organisms, as he had previously imagined, but that they can obtain their carbon from carbonates. These results were published in his fourth paper on nitrification (1891), and were communicated to the Chemical Society only a few days before Winogradski made a similar communication to the French Academy. Winogradski, however, had pushed the matter somewhat further, having obtained the organisms in bodily form, and having shown how they could be cultivated on solid media, a problem which had baffled Warington and other investigators. Warington, therefore, had to share his final hard-won success with another.

The practical results of nitrification in the soil were being investigated while the search for the organism was still in progress, and Warington began a long series of determinations of nitrates in the Rothamsted soil, the first results of which were published as a lecture given before the Society of Arts, for which he was awarded a silver medal.

When, in 1889, Lawes resigned his active control to the present Committee of Management, it was arranged that Warington should leave in the following January. Having, however, in the meantime, reached a very interesting stage in his work on the nitrifying organism, he stayed on at the laboratory till 1891, and succeeded in bringing the work on hand to a successful termination.

Although Warington's original work in agricultural chemistry was brought to a close on his severance from Rothamsted, much useful work remained for him to do. The Committee of Management appointed him American lecturer under the Lawes Trust, and he, consequently, proceeded to the United States to perform his functions. The six lectures which he there delivered dealt chiefly with the subject of nitrification, illustrated by his own work at Rothamsted. They were published by the United States Department of Agriculture.

On his return to England, Sir John Lawes invited him to carry out an investigation at his tartaric and citric acid factory at Millwall on the contamination of these acids by the lead of the vessels used in their preparation. This Warington undertook, and he succeeded in finding a method for obviating the evil. He obtained, in addition, an excellent method for the accurate volumetric determination of lead in the acid. This formed the subject of a communication to the Society of Chemical Industry in 1893, the last communication of any investigation made by him.

In 1894, he was appointed one of the examiners in Agriculture under the Science and Art Department, and in the summer of the same year he was elected Sibthorpian Professor of Rural Economy at Oxford for three years.

The papers, other than those on original investigations, which Warington wrote, are numerous, and are all characterised by a lucidity of expression and precision of argument which renders them specially valuable. One of the most useful of his writings is, undoubtedly, a little volume entitled "The Chemistry of the Farm." The amount of appreciation with which it has been received, and the good which it has done, may be measured by the fact that it is now in its fifteenth edition, and is accepted as the text book on the subject throughout the world, and as a model of what a text book of that sort should be.

Warington continued to reside in Harpenden till the end. His habits and tastes did not predispose him to take any active part in village management, but whenever he thought that his knowledge might be of service to the community he did not hesitate to give what assistance he could.

Educational or charitable work, however, always enlisted his sympathies, and engaged his active support; whilst his strong religious convictions, guided by his clear judgment and absolute sincerity, rendered his church and philanthropic work peculiarly valuable. He certainly had an unusually high sense of public duty, and, persistently throughout life, did what he could to make his fellow-creatures better and happier. Missionary work always held a prominent place in his heart, as also did the training of the young, whether in religious or secular subjects; and, during the last few years of his life, much of his time and care was devoted to the Church day-schools. He was greatly interested in all work amongst the poor and needy, and was a liberal supporter of any organised charity which appealed to his judgment. Partly owing to his isolated boyhood and youth, and partly to his lack of robust health, life went harder with him than it otherwise would have done, for the characteristics thus developed stood in his way, and often prevented his gaining the sympathy and appreciation which he was so ready to give to others.

Warington was elected to the Chemical Society in 1863, and to the Royal Society in 1886. He served for two periods on the Council of the Chemical Society, and for one period as vice-president. For many years he was on the library committee of this Society, and did much useful work for the Fellows during the reorganisation and cataloguing of the books. For this, his extensive acquaintance with chemical literature rendered him specially fitted.

Warington was married twice. His first wife was a daughter of G. H. Makins, F.R.C.S., formerly chief Assayer to the Bank of England, and one of the Court of Assistants at the Society of Apothecaries. His second wife was a daughter of Dr. F. R. Spackman, who had for many years been medical practitioner at Harpenden. He has left five daughters by his first wife. In 1906, his health gave way, and he had a serious illness which necessitated a very difficult and dangerous operation. For this he prepared with singular equanimity and courage. The operation was successful; but though he nominally recovered from it, he never regained his strength, and, eleven months afterwards (March 20, 1907), he passed away.

With the death of Warington, the first generation of great Rothamsted workers is brought to an end. Their work, whether published in the form of independent communications, or as joint productions, constitutes one great whole, of which the various parts are beautifully correlated and interdependent. It has placed agricultural science on an altogether different basis from that which it previously occupied, and the institution which gave birth to it has served as the prototype for similar institutions throughout the whole civilised world. The three men to whom we owe these results—Lawes, Gilbert and Warington—devoted their whole lives and energies to the work, and only those who are acquainted with the difficulties attendant on co-operation in this case can appreciate the devotion to science which was required to master these difficulties. All three workers now lie at rest in the same quiet country churchyard, their combined work in the cause of scientific agriculture forming the most fitting and enduring monument of their labours, for its importance becomes every day more and more evident with the development of the superstructure which is being raised upon it.

P. S. U. P.

WALTER FRANK RAPHAEL WELDON. 1860—1906.*

Walter Frank Raphael Weldon, the elder son of the late Walter Weldon, F.R.S., was born at Suffolk Villa, Highgate. We have no record if he attended a school there. When his parents removed to Putney he had, as tutor, a neighbouring clergyman. In 1873 he was sent to a boarding-school at Caversham, where he remained not quite three years, and from which, after some months of private study he matriculated, in 1876, in the University of London. In October of that year we find him at University College taking classes in Greek, English, Latin, and French, with two courses of Pure Mathematics. In the summer term of 1877, physics and applied mechanics were studied. During this whole session he also attended Daniel Oliver's general lectures on botany and Ray Lankester's on zoology. Later in the Christmas vacation of 1879, after he had gone up to Cambridge, he was for some weeks under Ray Lankester, who set him to work out the structure of the gills of the mollusc "Trigonia."

In the autumn of 1877 he transferred himself to King's College, where he stayed for two terms, attending classes in chemistry, mathematics, physics, and mechanics, besides the zoology course of A. H. Garrod and the biology of G. F. Yeo. Divinity under Barry, at that time compulsory, was also taken. At this time Weldon had the medical profession in view. Though only entered on the Register of Medical Students on July 6, 1878, there can be no question that his course, on the whole, was directed towards the Preliminary Scientific Examination of the London M.B. This examination he took in December, 1878, after he had gone up to Cambridge; he was coached for it by T. W. Bridge, now Professor of Zoology in Birmingham, but he had already completed the bulk of the work in his London courses. With the Preliminary Scientific, Weldon's relation with London University ceased. In 1877 he attended the Plymouth Meeting of the British Association, and there he was generally to be found in Section D.

The presence of a life-long friend, who had already gone to Cambridge, was, at least, one of the causes which led to Weldon's entering himself as a bye-term student at Cambridge; and probably his choice of St. John's College was due to Garrod's influence. He was admitted on April 6, 1878, as a pupil of the Rev. S. Parkinson, D.D.

At Cambridge Weldon soon found his work more specialised, and he rapidly came under new and marked influences. Under the inspiration of Balfour, Weldon's thoughts turned more and more to zoology, and the medical profession became less and less attractive. During the years 1879 and 1880, he worked steadily for his Tripos; in the first year he was given an exhibition at St. John's. In the second year a little original investigation on beetles was started; in May he took, for a month, Adam Sedgwick's place, and demonstrated for Balfour.

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* This notice is abstracted (by A. E. S.) from the much longer biography in 'Biometrika,' written by Professor Karl Pearson, with some help from Mr. A. E. Shipley.

The Tripos work was continued, in spite of ill-health, till the Easter of 1881, when Weldon was unable to enter for the college scholarship examinations. By the influence of Francis Balfour, however, Weldon's real ability was recognised, and a scholarship was awarded to him. At the very start of his Tripos examination, his only brother, Dante Weldon, who had joined Peterhouse, died suddenly of apoplexy. It says much for Weldon's self-control that the terrible shock of his brother's death did not interfere with his place in the first class of the Natural Sciences Tripos. A few weeks later a second bereavement befell him, when his mother passed away. These trials, followed by Balfour's untimely death in the following year, and by the early death of his own father a few years later, left their indelible impresses upon him.

With the Tripos, Weldon's "Lehrjahre" closed, and, as his nature directed, the "Wanderjahre" began without any interval of rest. Immediately after his Tripos, Weldon started for Naples to work at the Zoological Station. The charm of Balfour's personality had aroused the affection of all who attended his classes, and had awakened a keen desire to follow in his footsteps. In those days the stimulus given by Darwin's writings to morphological and embryological researches was still the dominating factor amongst zoologists, and Weldon threw himself at first with ardour into the effort to advance our knowledge by morphological methods. In Naples he began his first published work, a "Note on the early Development of *Lacerta muralis*," and at the same time did much miscellaneous work on marine organisms.

In September he was back in England at the Southampton Meeting of the British Association. Adam Sedgwick, who had succeeded to the teaching work of Francis Balfour, now invited Weldon to demonstrate for him. Thus, the winter found Weldon in Cambridge again, and from Sedgwick's laboratory was issued the next piece of work: "On the Head-kidney of Bdellostoma, with a suggestion as to the Origin of the Suprarenal Bodies," and he followed the subject up in the next year by publishing his paper "On the Suprarenal Bodies of Vertebrates."

On March 14, 1883, the anniversary of his parents' wedding-day, Weldon married Miss Florence Tebb, the eldest daughter of William Tebb, now of Rede Hall, Burstow, Surrey.

After the death on January 14, 1883, of W. A. Forbes, a Fellow Johnian, Weldon for four months—June 15 to October 15—acted as *locum tenens* for the Prosector at the Zoological Gardens, London, and during that time he read the following papers before the Zoological Society:—"On some points in the Anatomy of Phœnicopterus and its Allies"; a "Note on the Placentation of *Tetraceros quadricornis*"; and "Notes on *Callithrix gigot*."

In the following year (1884) the paper above referred to on the development of the suprarenal bodies was published in the Royal Society 'Proceedings.' On November 3 of the same year, Weldon was elected to a Fellowship at St. John's College, and was shortly afterwards appointed University Lecturer in Invertebrate Morphology. About this time he took a permanent home at No. 14, Brookside, which soon became a centre for Cambridge workers on biology.

On his return to Cambridge in November, 1884, Weldon had taken up again his invertebrate work. His next Memoir, "On *Dinophilus gigas*," dealt with the anatomy and affinities of Dinophilus, at that time a very little known Annelid.

The next few years of Weldon's life were more active than ever. He had now given up coaching, and as he only needed to be in Cambridge two terms of the year, travel and research could occupy the time from the beginning of June to January. On May 8, 1885, he gave his first Friday evening lecture at the Royal Institution on "Adaptation to surroundings as a factor in Animal Development." No report of this lecture was published in the 'Proceedings,' but there are those who still remember the impression caused by the youthful lecturer of twenty-five years of age. Weldon was an adept in lecturing to classes of University students; it brought out all his force and enthusiasm as a teacher. As a writer in the 'Times' (April 18, 1906) says: "Seldom is it given to a man to teach as Weldon taught. He lectured almost as one inspired. His extreme earnestness was only equalled by his lucidity. He awoke enthusiasm even in the dullest, and he had the divine gift of compelling interest. In the University Lecture room he always impressed his hearers with the importance of his topic. You could not listen to him lecturing on a flame-cell or on the variations in the carapace of *Pandalus annulicornis* without sharing his intense conviction of the importance of the matter in hand. He aroused a consciousness in his students that things were worth studying for their own sake, apart from their examination value."

In July, 1886, Weldon crossed to America, and visited the Bahamas to collect. From his headquarters in the Bahamas, he went with two friends to North Bimini, in the Gulf Stream, and made considerable collections, but his published results were confined to "*Haplodiscus piger;* a new Pelagic Organism from the Bahamas," and a "Preliminary note on a *Balanoglossus* Larva from the Bahamas." Working at the *Balanoglossus* material in 1887, he found that his results differed from those reached by Professor Sprengel. He accordingly went to Giessen at Easter, and finally handed over to Professor Sprengel the whole of his *Balanoglossus* material. During the Lent and May terms he gave a course of lectures on Economic Entomology to the forestry students at the Royal Indian Engineering College, Coopers Hill.

In 1888, the buildings of the Marine Biological Laboratory in Plymouth were nearly completed, and to the Marine Biological Association Weldon gave both time and sympathy during the rest of his life. His annual visits of inspection to their second Laboratory at Lowestoft during the last few years were always a great pleasure to him. Lent and May terms, 1888, were spent as usual in Cambridge, but June to December were given up to Plymouth, with a brief Christmas holiday in Munich. And here we must note the beginning of a new phase in Weldon's ideas. His thoughts were distinctly turning from morphology to problems in variation and correlation. He has left on record the nature of the problems he was proposing to himself at this time, and they are summed up as follows:—

(1) The establishment of a new set of adult characters leading to the evolution of a new family has always been accompanied by the evolution of a new set of larval characters leading to the formation of a larval type peculiar to the newly established family; the two sets of characters having as yet no demonstrable connection one with the other.

(2) The evolution of the adult and that of the larval characters peculiar to a group advance *pari passu* one with the other, so that a given degree of a specialisation of adult characters on the parts of a given species implies the possession of a larva having a corresponding degree of specialisation and *vice versâ*.

The next year was to place in Weldon's hands a book—Francis Galton's "Natural Inheritance," by which one avenue to the solution of such problems, one quantitative method of attacking organic correlation, was opened out. From this book as their source sprang two notable friendships and the whole of the biometric movement, which so changed the course of his life and work. In 1889, also, another change came. Weldon found that his dredging and collecting work separated him from his books for half his time. Accordingly, he applied for a year's leave from Cambridge, and he and his wife settled down in a house of their own at Plymouth. This period of hard work lasted through 1890, and was broken only by flying visits to Dresden in September and at Christmas, 1889, and an autumn visit in 1890 to Chartres and Bourges. The intellectual development and the experience and knowledge gained in this period were far more important than the mere published work would indicate. In 1889, Weldon investigated the nature of the curious enlargement of the bladder associated with the green, or excretory, glands in certain Decapod Crustacea, and published in October of the same year his paper of "The Cœlom and Nephridia of *Palæmon servatus*." The result of his investigation was to confirm "the comparison so often made (by Claus, Grobben, and others) between the glomerulus of the vertebrate kidney and the end-sac of the Crustacean green gland." A little later, June, 1891, he published the results of more extended researches in this field in what proved to be his last strictly morphological paper. It was entitled: "The Renal Organs of certain Decapod Crustacea." In this he showed that in many Decapods spacious nephro-peritoneal sacs "should be regarded rather as enlarged portions of the tubular system . . . than as persistent remnants of a 'cœlomic' body cavity, into which the tubular nephridia open."

One further paper of a year later may be best referred to here, Weldon's only piece of work on invertebrate embryology, "The formation of the Germ Layers in *Crangon vulgaris*." This contains a clear account of the early stages of segmentation, and the building up of the layers of the shrimp, illustrated by excellent figures. And here it may be mentioned that his power with the pencil was not that of the mere draughtsman, accurate in detail but often lifeless; he was an artist by instinct, and he had the keenest pleasure in drawing for its own sake.

December, 1890, closed the Cambridge work; Weldon now succeeded Ray Lankester in the Jodrell Professorship at University College, London. In June he had been elected a Fellow of the Royal Society.

It has been seen that the years between Weldon's degree and his first professoriate were years of intense activity. He was teaching many things, studying many things, planning many things. His travels perfected his linguistic powers, and his fluency in French, Italian, and German was soon remarkable.

A word must here be said as to the transition which took place during the "Wanderjahre" in Weldon's ideas. He had started, as most of the younger men of that day, with an intense enthusiasm for the Darwinian theory of evolution, but he realised to the full that the great scheme of Darwin was only a working hypothesis, and that it was left to his disciples to complete the proofs, of which the master had only sketched the outline. Naturally he turned first to those methods of proof, morphological and embryological, which were being pursued by the biological leaders of the period, and it was only with time that he came to the conclusion that no great progress could be attained by the old methods. We have already seen that even before the appearance of "Natural Inheritance," his thoughts were turning on the distribution of variations and the correlation of organic characters. He was being led in the direction of statistical inquiry. The full expression of his ideas is well given in the first part of the "Editorial," with which 'Biometrika' started:—

> "The starting point of Darwin's theory of evolution is precisely the existence of those differences between individual members of a race or species which morphologists for the most part rightly neglect. The first condition necessary, in order that any processes of Natural Selection may begin among a race, or species, is the existence of differences among its members; and the first step in an inquiry into the possible effect of a selective process upon any character of a race must be an estimate of the frequency with which individuals, exhibiting any degree of abnormality with respect to that character, occur. The unit, with which such an enquiry must deal, is not an individual but a race, or a statistically representative sample of a race; and the result must take the form of a numerical statement, showing the relative frequency with which various kinds of individuals composing the race occur."

It was Francis Galton's "Natural Inheritance" that first indicated to Weldon the manner in which the frequency of deviations from the type could be measured.

In Plymouth, 1890, Weldon started his elaborate measurements on the Decapod Crustacea, and soon succeeded in showing that the distribution of variations was closely like that which Quetelet and Galton had found in the case of man.

His paper "The Variations occurring in certain Decapod Crustacea. I. *Crangon vulgaris*" was, as far as we know, the first to apply the method of Galton to other zoological types than man. In this paper the author showed that different measurements made on several local races of shrimps give frequency distributions closely following the normal or Gaussian law. In his next paper, "On certain correlated Variations in *Crangon vulgaris*," he calculated the first coefficients of organic correlation, *i.e.*, the numerical measures of the degree of interrelation between two organs or characters in the same individual. It is quite true that the complete modern methods were not adopted in either of these papers, but we have for the first time organic correlation coefficients—although not yet called by that name—tabled for four local races. These two papers are epoch-making in the history of the science, afterwards called Biometry.

It is right to state that Weldon's mathematical knowledge at this period was far more limited than it afterwards became. The first paper was sent to Francis Galton as referee, and was the commencement of a life-long friendship between the two men. With Galton's aid the statistical treatment was remodelled, and considerable modifications made in the conclusions.

The defect in mathematical grasp, which Weldon had realised in his first paper, led him at once to seek to eliminate it. He set about increasing his mathematical knowledge by a thorough study of the great French writers on the calculus of probability. He did not turn to elementary text-books, but with his characteristic thoroughness went to the fountain head, and he thus attained a great power of following mathematical reasoning, and this power developed with the years. He had, moreover, a touch with observation and experiment rare in mathematicians. In problems of probability he would start experimentally and often reach results of great complexity by induction. From 1890 onwards, his knowledge, theoretical and experimental, of the theory of chances increased by bounds.

Weldon's work at University College commenced in 1891. The house in Wimpole Street was taken and, if possible, life became more intense. In October came the college inaugural lecture for the session, on the subject of the statistical treatment of variation. This year and the next were strenuous years in calculating. The Weldons toiled away at masses of figures, doing all in duplicate. At Easter, 1892, they went to Malta and Naples, and the summer was spent over crab-measurements at the Zoological Station in the latter city, and the first biometric crab paper "On certain correlated Variations in *Carcinus mœnas*" was issued later in the year. This paper confirms on the shore crab the results already obtained on the common shrimp. The distributions of characters are closely Gaussian, with the exception of the relative frontal breadth, which the author considered dimorphic in Naples. He does not refer to this fact in his memoir. As for shrimps the correlations again came out closely alike for the Plymouth and Naples races. Weldon was not dogmatic on the point; he considered the constancy as at least an "empirical working rule."

To the biometrician, perhaps, the most interesting committee with which Weldon was associated in his later years was that which came to be called the Royal Society Evolution (Animals and Plants) Committee. His papers on variation and correlation in shrimps and crabs had brought him closely into touch with Francis Galton, and both were keenly interested in the discovery of further dimorphic forms such as had been suggested by the frontal breadths of the Naples crabs. Weldon was full also of other ideas ripe for investigation. He had started his great attempt at the measurement of a selective death-rate in the crabs of Plymouth Sound; experiments on repeated selection of infusoria were going on in his laboratory; he was gathering an ardent band of workers about him, and much seemed possible with proper assistance and that friendly sympathy which was ever essential to him. As a result of an informal conference held at the Savile Club towards the end of 1893, it was decided to ask the Royal Society to establish a Committee "for the purpose of Conducting Statistical Enquiry into the Variability of Organisms," and such a Committee was early in 1894 constituted by the Council, with Francis Galton as Chairman, and Weldon as Secretary, the Committee being entitled; "Committee for conducting Statistical Inquiries into the Measurable Characteristics of Plants and Animals." The use of the words statistical and measurable, somewhat narrowly, but accurately, defined the proposed researches of the Committee. It went on until 1897, with the same members, the same title and scope. Looking back on the matter now, one realises how much Weldon's work was hampered by the Committee. It is generally best that a man's work should be published on his own responsibility, and when he is a man of well-known ability and established reputation, grants in aid can always be procured. In this case Weldon had a sympathetic committee, but the members were naturally anxious on the one hand for the prestige of the Society with whose name they were associated, and secondly, they were desirous of showing that they were achieving something. Both conditions were incompatible with tentative researches such as Biometry then demanded. Trial and experiment were peculiarly needful in 1893; the statistical calculus itself was not then even partially completed; biometric computations were not reduced to routine methods, and the mere work of collecting, observing, experimenting, and measuring was more than enough for one man. Weldon with his "volume of life" was eager to do all these things, and run a laboratory with perhaps sixty students as well.

The "Attempt to Measure the Death-rate due to the Selective Destruction of *Carcinus mœnas,* with respect to a Particular Dimension," formed the first report of the Committee, and was presented to the Royal Society in November, 1894. Weldon's general project in this case was novel at the time, it consisted in determining whether the death-rate is correlated with measurable characters of the organism, or, as he himself puts it, "in comparing the frequency of abnormalities in young individuals at various stages of growth with the frequency of the same abnormalities in adult life, so as to determine whether any evidence of selective destruction during growth could be discovered or not."

Looking back now on Weldon's paper of 1894, one realises its great merits: it formulated the whole range of problems which must be dealt with biometrically before the principle of selection can be raised from hypothesis to law. Almost each step of it suggests a mathematical problem of vital importance in evolution, which has since been developed at length, or still awaits the labour of the ardent biometrician.

Unfortunately the paper, as well as the suggestive "Remarks on Variation in Animals and Plants" with its memorable words: "The questions raised by the Darwinian hypothesis are purely statistical, and the statistical method is the only one at present obvious by which that hypothesis can be experimentally checked," fell on barren soil. A further instructive report on the growth at two moults of a considerable number of crabs was made to the Committee in 1897, but appears never to have been published. Later, an account of work on Natural Selection in crabs was given by Weldon in his Presidential Address to the Zoological Section of the British Association, Bristol, 1898. In the paper just mentioned, after several years of discouragement and much hard labour, he succeeded in demonstrating that natural selection was really at work, and further that it was at work at a very sensible rate. The labour involved was excessive. One "crabbery" consisted of 500 wide-mouthed bottles, each with two syphons for a constant flow of sea water. Each crab had to be fed daily and its bottle cleaned. But in the autumn a rest came. The British Association Address was written and Weldon thoroughly enjoyed his presidency of Section D at Bristol.

It may not be out of place here to note the great aid Weldon's artistic instinct and literary training gave to his scientific expression. His papers are models of clear exposition, his facts are well marshalled, his phraseology apt, his arguments concise, and his conclusions tersely and definitely expressed. The result, however, was not reached without much labour. There was never any artificial brilliancy introduced in the process; rhetoric in the service of science was intolerable to Weldon. His was simply an attempt to choose the suitable form and the right words for a given purpose. It was comparable with his sense of sound, with his extraordinary gift of appreciating and reproducing the exact intonation of a foreign tongue.

Considerable changes were soon to take place in his environment and scheme of work. Lankester had been appointed Director to the British Museum (Natural History), and in February, 1899, Weldon succeeded him in the Linacre Professorship at Oxford. In the February of 1897 the Royal Society Evolution Committee received a large increase of membership; it ceased henceforth to "conduct statistical inquiries into the measurable characteristics of plants and animals," and became transformed into an Evolution (Plants and Animals) Committee and Weldon and the biometric members ultimately withdrew from it.

During the eight years of his London professoriate Weldon's development was great; he became step by step a sound mathematician, and gained largely in his power of clear and luminous exposition. His laboratory was always full of enthusiastic workers, and over forty memoirs were published by his students. His removal from the London field of work, while an incalculable loss to his colleagues, was not without its compensation to his nearest friends. They knew that the life of the last few years had been one of great tension, that Weldon's time had been too much encroached upon by committee work, that the separation between the locus of his teaching and of his research work was very undesirable; that even the social life of London involved too much expense of energy. He was a child of the open air and the breezes, and it was hoped that he might have more of them, if not in lowland Oxford, at least on the hills around. There was space and air too for the experimental work that had been so cramped in Gower Street. The *Daphnia* studies, which had occupied so much energy under unfavourable conditions in London, were at once resumed on broader lines in the ponds and ditches round Oxford. With a basket of bottles attached to his cycle handle, and a fishing creel, filled with more bottles, on his back, the Linacre Professor might be met even as far as the Chiltern Hills, collecting not only *Daphnia*, but samples of the water in which they lived. His University College work had shown him how widely *Daphnia* are modified by their chemical and physical environment, and how this modification is largely due to selection. There exist elaborate drawings of the *Daphnia* from the Oxfordshire ponds, indicating their differentiation into local races, with notes on the peculiarity of their habitat and the chemical constitution of the water.

A study of Kobelt's "Studien zur Zoogeographie," 1897–9, led him later to take up the same problem with regard to land-snails. What is the meaning of the slight but perfectly sensible differences in type to be found in shells from adjacent valleys or even from different heights of the same mountain? Weldon attacked the problem in his usual manner; he spent two Christmas vacations collecting Sicilian snails of the same species, from habitats extending over a wide area, the local environments were described, and the snails were often photographed with their immediate surroundings. Innumerable shells were brought back to Oxford, and the Professor delighted to discourse on the significant differences in local type, and yet the gradual change of type to type from one spot to another. No rapidly made measurement on the outside of the shell would satisfy him; the shell must be carefully ground down through the axis, and the measurements must be made on the section thus exposed. Perhaps four or five snail shells could be ground and measured in a day, and at the time of his death not more than a few hundred of the Sicilian thousands had even been ground.

But these attempts to get to the kernel of selection in its action on local races were far from occupying the whole of Weldon's thoughts in these early days. In conjunction with his assistant, Dr. E. Warren, he had commenced at University College his first big experimental investigation into heredity. The characters dealt with consisted of the number of scales in particular colour patches upon certain pedigree moths, and the work of counting these was very laborious. In the course of three years, many hundreds of pedigree moths were dealt with, and the observations were reduced. But no definite inheritance at all of the character selected for consideration was discovered. Weldon, apparently thought that there had been some fatal mistake in the selection of pairings, and undoubtedly, in some cases, parents of opposite deviation had been mated, so that a rather influential negative assortative mating resulted. But from other series of pedigree moth data, it seems probable that there is some special feature in heredity in moths, or possibly in those that breed twice in the year, and that the vast piece of work which Weldon and Warren undertook in 1898—1901 may still have its lesson to teach us.

In these three first years at Oxford, Weldon's intellectual activity was intense. To the pedigree moth experiments was added, in the summer of 1900, an elaborate series of Shirley Poppy growings, 1250 pedigree individuals being grown and tended in separate pots; Weldon's records were the most perfect of those of any of the co-operators, and his energy and suggestions gave a new impetus to the whole investigation. They were ultimately published in 'Biometrika' under the title, "Co-operative Investigations on Plants, I. On Inheritance in the Shirley Poppy." As Weldon himself expressed it, the moths and poppies meant "a solid eight hours daily of stable-boy work through the whole summer and through the Easter vacation, with decent statistical work between." After the Shirley Poppies were out of hand in the summer of 1900, the Weldons went to Hamburg and thence to Plön. The object of this visit was to collect *Clausilia* at Plön and Gremsmühlen for comparison with the race at Risborough. The same aim—the comparison of local races—led Weldon at Christmas to collect land-snails in Madeira. Thus he slowly built up a magnificent biometric collection of snail shells, *i.e.*, one sufficiently large to show, in the case of many local races of a number of species, the type and variability by statistically ample samples. Of this part of his work only two fragments have been published, "A First Study of Natural Selection in *Clausilia laminata*" (Montagu) and "Note on a Race of *Clausilia itala*" (von Martens). In the first of these memoirs he shows that two races of *C. laminata* exist, in localities so widely separated as Gremsmühlen and Risborough, with sensibly identical spirals, although no crossing between their ancestors can have existed for an immense period of time, and although there are comparatively few common environmental conditions. At the same time, while no differential secular selection of the spiral appears to have taken place during this period, there yet seems to be a periodic selection of the younger individuals in each generation, the variability of the spirals of the young cells being sensibly greater than of the corresponding whorls of adults. In other words, stability to the type is preserved by selection in each new generation. In the second memoir, Weldon sought for demonstration of a like periodic selection in the *C. itala* he had collected from the public walks round the Citadel of Brescia. He failed, however, to trace it, and was forced to conclude that *C. itala* is either not now subject to selective elimination for this character or is multiplying at present under specially favourable conditions at Brescia, or again, as both young and old were gathered in early spring, after their winter sleep, that elimination takes place largely during the winter, and "that individuals of the same length, collected in the autumn, at the close of their period of growth, might be more variable than those which survive the winter."

The problem of growth, to be studied only under conditions of captivity, possibly modifying the natural growth immensely, has made the crab investigation an extremely complex one. Weldon solved the difficulty by the brilliant idea that the snail carries with it practically a record of its youth. If the wear and tear of the outside of the shell to some extent confuses the record there, a carefully ground axial section will reveal by the lower whorls the infancy of the organism. Hence the days given to experimental grinding, the training in manipulation and the final success, and then the steady work, grinding and measuring a few specimens a day, till the necessary hundreds were put together; the laborious calculations not in the least indicated in the papers; and the illustration of how shells may be used—by those who will give the needful toil—to test the truth of the Darwinian theory.

On November 16, Weldon wrote:—

> "Do you think it would be too hopelessly expensive to start a journal of some kind? . . . .
>
> "If one printed five hundred copies of a royal 8vo. once a quarter, sternly repressing anything by way of illustration except process drawings and curves, what would the annual loss be, taking any practical price per number? . . . . If no English publisher would undertake it at a cheap rate, the cost of going to Fischer, of Jena, or even Engelmann would not be very great."

This was the first definite suggestion of the establishment of 'Biometrika.' On November 29, the draft circular, corresponding fairly closely to the first editorial of the first number, reached his co-editor from Oxford with the words: "Get a better title for this would-be journal than I can think of!" The circular went back to Oxford with the suggestion that the science in future should be called Biometry, and its official organ be 'Biometrika.' And on December 2, 1900, Weldon wrote;—

> "I did not see your letter yesterday until it was too late for you to have an answer last night. I like 'Biometrika' and the sub-title."

Thus was 'Biometrika' born and christened. The reply to circulars issued during December was sufficiently favourable to warrant further proceedings. By June of 1901 its publication through the Cambridge University Press had been arranged for, and the sympathetic help of the Syndics and the care given by the University Printers enabled us to start well and surmount many difficulties peculiar to a new branch of science. During the years in which Weldon was co-editor with Karl Pearson he contributed much, directly and indirectly, to its pages. He was referee for all essentially biological papers; and his judgment in this matter was of the utmost value. He revised and almost re-wrote special articles. He was ever ready with encouragement and aid when real difficulties arose.

Starting on October 16, 1900, and extending throughout the early 'Biometrika' letters to his co-editor, runs a flood of information with regard to Mendel and his hypothesis.

> "About pleasanter things: I have heard of and read a paper by one Mendel, on the results of crossing peas, which I think you would like to read. It is in the 'Abhandlungen des naturforschenden Vereines in Brunn' for 1865. I have the R.S. copy here, but I will send it to you if you want it."—(October 16, 1900.)

Then follows a résumé of the first of Mendel's memoirs, and for months the letters—always treatises—are equally devoted to snails, Mendelism, and the basal things of life.

The earlier part of 1901 was chiefly occupied by snails, but a new factor had come into Weldon's many-sided occupations. It was settled that 'Biometrika' should have in an early number a critical bibliography of papers dealing with statistical biology. Weldon undertook the task of preparing it, as his study of Mendel had led him to a very great number of such papers dealing with inheritance, and the section on Heredity was to be published first. Like all his projects, it was to be done in so thorough and comprehensive a manner that years were required for its completion. A very full list of titles was formed, especially in the Inheritance section, and many of the papers therein were thoroughly studied and abstracted. But such study meant with him not only grasping the writer's conclusions, but testing his arithmetic and weighing his logic. Thus Weldon's note on "Change in Organic Correlation of *Ficaria ranunculoides* during the Flowering Season," arose from this bibliographical work and the erroneous manner in which he found Verschaffelt and MacLeod dealing with correlation. A further result of this work was that his confidence in the generality of the Mendelian hypotheses was much shaken. He found that Mendel's views were not consonant with the results formulated in a number of papers he had been led to abstract, and that the definite categories used by some Mendelian writers did not correspond to really well-defined classes in the characters themselves.

To those who accept the biometric standpoint, that, in the main, evolution has not taken place by leaps, but by continuous selection of the favourable variation from the distribution of the offspring round the ancestrally fixed type, each selection modifying *pro rata* that type, there must be a manifest want in Mendelian theories of inheritance. Reproduction from this standpoint can only shake the kaleidoscope of existing alternatives; it can bring nothing new into the field. To complete a Mendelian theory we must apparently associate it for the purposes of evolution with some hypothesis of "mutations." The chief upholder of such an hypothesis has been de Vries, and Weldon's article on "Professor de Vries on the Origin of Species" was the outcome of his consideration of this matter. During the years 1902 and 1903 an elaborate attempt was made to grow the numerous sub-races of *Draba verna*, with the idea that they might throw light on mutations. The project failed, largely owing to difficulties in the artificial cultivation of some of the species. But for a time all other interests paled before *Draba verna*.

A study of the work of von Guaita had convinced Weldon, early in 1901, that the cross between the European albino mouse and the Japanese waltzing mouse was not one which admitted of simple Mendelian description. In May, 1901, his letters contain inquiries as to Japanese mice dealers. During the summer and autumn the collection of Japanese mice was in progress. These mice were to be bred to test the purity of the stock; during December about forty does had litters, and pure breeding went on until the autumn of 1902, when hybridization commenced. The work on these mice was for two years entrusted to Mr. A. D. Darbishire, but the whole plan of the experiments, the preparation of the correlation tables, and the elaborate calculations were in the main due to Weldon. On Mr. Darbishire's leaving Oxford, Weldon again resumed personal control of the actual breeding arrangements, and from some second hybrid matings carried on the work to the sixth hybrids' offspring. The work was nearing completion at his death, and through the energy of Mr. Frank Sherlock, the skins of the 600 pedigree mice forming the stud at that time have been dressed and added to those of the earlier generations. Weldon had this work much at heart, and his letters during 1904 and 1905 give many indications of the points he considered demonstrated. The experimental part of the work would have been nearly completed had not his whole thought and energy been directed from November, 1905, into another channel.

In the summer 'Biometrika' was edited from Bainbridge in Wensleydale, and the co-editors cycled to the churchyards of the Yorkshire dales, collecting material for their joint paper "On Assortative Mating in Man" (34). From Bainbridge, the Weldons went to the British Association meeting at Belfast, where an evening lecture on Inheritance was given. At Christmas came one of the above-mentioned visits to Palermo to collect Sicilian snails.

In the spring of 1903, Weldon was busy, as were the whole available members of the biometric school, in studying the influence of environment and of period of season on the variation and correlation of the floral parts of Lesser Celandine.

> "Give my love to the Brethren who are co-operating in the matter of Celandines, and beseech them to make a better map of their country than the enclosed."—(Oxford, February 23, 1903.)

Weldon threw his whole energy and love of minute exactitude into the task, and his letters are filled with an account of the almost daily changes in the type and variability of the Celandine flowers from his selected stations. The result of this enquiry was the collection of an immense amount of data showing that environment and period in the flowering season affected the flower characters to an extent comparable with the differences attributed to local races. At Easter of 1903 a series of mishaps prevented the common holiday, but this was more than compensated for by the summer vacation. The Weldons started with a sea trip to Marseilles and back. They then returned to Oxford, in order that work on the article "Crustacea" for the Cambridge Natural History might be carried on, and that an eye might be kept on the mice. The data on assortative mating in man collected in the previous year were reduced and a joint paper sent to press; the immense amount of calculation and reduction involved in the mouse-paper was got through; a joint criticism of Johannsen's "Ueber Erblichkeit in Populationen und in reinen Linien" was written by the co-editors, under the title "Inheritance in *Phaseolus vulgaris*," and a joint study was made, at Weldon's suggestion, of the relationship between Mendelian formulæ and the theory of ancestral heredity. It was shown that there was no essential antagonism between the two methods of approaching the subject, and the results were published ultimately at Part XII of the "Mathematical Contributions to the Theory of Evolution," Weldon persistently declining to allow it to appear as a joint memoir, because he had taken no part in certain portions of the more complicated algebraic analysis. Christmas found the Weldons in Palermo on the snail quest. His letters thence to his co-editor teem with the freshness of the sky and the joy of open-air work.

> "Out between five and six, in the dark, without any breakfast, sunrise up in the hills, a day's tramp on a piece of bread and a handful of olives, and home at seven, laden with snails. Then after dinner to clean the beasts. That is not work, and it makes one very fit, but one gets tired enough to sleep when the snails are cleaned."

At the beginning of 1904 the work on the Brescia *Clausilia* was in progress, the mice were multiplying after their kind, and Weldon's thoughts were turning more and more to a determinal theory of inheritance, which should give a simple Mendelism at one end of the range and blended inheritance at the other. The summer found the Pearsons twelve miles from Oxford, at Cogges, near Witney, and the Galtons, twenty miles further, at Bibury; there was much cycling to and fro, and the plan of a new book by Weldon on Inheritance was drafted, and some of the early chapters were written.

The book on Inheritance occupied most of the remainder of the year, and to aid it forward and help those of us who were not biologists to clearer notions, Pearson suggested to Weldon a course of lectures in London to his own group of biometric workers. The project grew, other departments of the College desired to attend, and ultimately the lectures were thrown open to all members of the University and even to the outside public. The lecturer had a good audience of more than a hundred, and enjoyed the return to his old environment.

The letters of Weldon to both Francis Galton and Pearson during the years 1904 and 1905 are full of inheritance work, the details of the great mice-breeding experiment, the statement and the solution, or it might be the suggested solution, of nuclear problems leading to detrimental theories of inheritance. Occasionally, there would be a touch of conscience, and the drawings for the Crustacea would be pressed forward :—

> "I ought to give my whole time to the 'Cambridge Natural History' for a while. They had been very good to me, and I have treated them more than a little badly. I am rather anxious to get them off my conscience."—(Oxford, February 15, 1905.)

But only the chapter on "Phyllopods" was completed, figures and all, and was set up in type. Many figures were prepared for other parts; beautiful things, which gave Weldon not only scientific but artistic pleasure, he had made, but the text remains a mere fragment. In the same way but little was absolutely completed of the article on "Heliozoa" for Lankester's "Natural History." It was not Weldon's biometric friends who kept him from these tasks, but solely his own intense keenness in the pursuit of new knowledge.

The fascination of inheritance problems kept him, however, for months at a time at the Heredity book. At Exeter, 1905, he went to Ferrara because that place had a university, and as such must have a library, where work could be done. The contents of the library were perfectly mediæval, a characteristic appropriate in the castle, but hardly helpful in heredity. Still, portions of the manuscript came to England for comment and criticisms, and we were hopeful that the end of the year would see the book completed.

It must not be thought for a moment that Weldon was desultory in his work. As Sir Ray Lankester says in a letter: "His absolute thoroughness and unstinting devotion to any work he took up were leading features in his character." He pursued science, however, for sheer love of it, and he would have continued to do so had he been Alexander Selkirk on the island with no opportunity of publication and nobody to communicate his results to. He never slackened in the energy he gave to scientific work, but having satisfied himself in one quest he did not stay to fill in the page for others to read; his keen eye found a new problem where the ordinary man saw a cow-pasture, or a dusty hedgerow, and he started again with unremitted ardour to what had for himself the greater interest.

In the summer the Pearsons were at East Ilsley, some seventeen miles from Oxford, and there was cycling out several times a week; there was steady joint work on the determinantal theory of inheritance as outlined by Weldon, which, it is to be hoped, is sufficiently advanced to be completed and published. He had in August, 1905, given to the Summer Meeting of the University Extension in Oxford a lecture on "Inheritance in Animals and Plants," and this had taken up some of his energy during the summer vacation. On the whole, however, he worked persistently at the Inheritance book.

It cannot be denied that those who were often with Weldon during the last two years were occasionally anxious on his account. The pace at which he worked had been too great—but at no time was it definitely realised that there was cause for immediate alarm. His intellectual activity was never apparently diminished, and his long cycling rides were maintained to the end; though an occasional, but never long persistent, lack of the old joyousness of life was noticeable.

In November, 1905, Weldon was unfortunately taken off from the work on his Inheritance book by the presentation to the Royal Society of a paper by Captain C. C. Hurst, "On the Inheritance of Coat-Colour in Horses." He had had no proper summer holiday, but he threw himself nine hours a day into the study of "The General Studbook."

> "I can do nothing else until I have found out what it means . . . The question between Mendel and Galton's theory of Reversion ought to be answered out of these. Thank God, I have not finished that book. There must be a chapter on Race Horses!"

He promised to communicate a note to the Society involving details of his inquiry. This was done on January 18, 1906, in a "Note on the Offspring of Thoroughbred Chestnut Mares."

> "The object of the present note is partly to fulfil my promise and partly to call attention to certain facts which must be considered in the attempt to apply any Mendelian formula whatever to the inheritance of coat-colour in race horses."

Here it can only be said that he took up the subject with his usual vigour and thoroughness. But he was overworked and overwrought, and a holiday was absolutely needful. He went to Rome, but the volumes of the Studbook went with him. His letters are filled with Studbook detail till Easter, with hardly a reference to anything else. Re-reading them now, one sees how this drudgery, with no proper holiday, told on him. Hundreds of pedigrees were formed, and a vast amount of material was reduced. At Easter, he and his wife went to the little inn at Woolstone, at the foot of the White Horse

Hill, and his co-editor came down later to Longcot, a mile away, for the joint vacation. Weldon, still hard at work on the Studbooks, was intellectually as keenly active as of old; and was planning the lines of his big memoir on coat-colour in horses, and showing how they illustrated the points he had already found in the mice.

This extraordinary mental activity was now telling upon a constitution never very robust, but the end came with startling suddenness. A day or two of slight illness at Woolstone, which, as usual, he made nothing of, was followed by a visit alone to London on Wednesday, April 11. Here he was taken seriously ill, and within a few hours he died of pneumonia, on Good Friday, April 13, 1906.

So passed away, not unfitly—for it was without any long disabling illness and in full intellectual vigour—a man of unusual personality, one of the most inspiring and loveable of teachers, the least self-regarding and the most helpful of friends, and the most generous of opponents.

And lastly, as to Science; What will his place be? The time to judge is not yet. Much of his work has still to be published, and this is not the occasion to indicate what Biometry has already achieved. The movement he aided in starting is but in its infancy. It has to fight not for this theory or that, but for a new method and a greater standard of logical exactness in the science of life. To those who condemn it out of hand, or emphasise its slightest slip, we can boldly reply, "You simply cannot judge, for you have not the requisite knowledge." To the biometrician, Weldon will remain as the first biologist who, able to make his name by following the old tracks, chose to strike out a new path—and one which carried him far away from his earlier colleagues. It is scarcely to be wondered at if those he joined should wish to see some monument to his memory; for he fell, the volume of life exhausted, fighting for the new learning.

OBITUARY NOTICES

OF

FELLOWS DECEASED.

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HENRY BAKER TRISTRAM, 1822—1906.

The Rev. Henry Baker Tristram, long familiarly known to naturalists all over the world as Canon Tristram, was born on May 11, 1822, at Eglingham, near Alnwick, of which large parish his father was vicar. He received his early education at Durham School, and passed to Lincoln College, Oxford, where he graduated in 1844, taking a second class in classics. In the following year he was ordained Deacon, and shortly afterwards became Curate of Morton Bishop. He had not been long engaged in his clerical duties when he developed such signs of a weak chest that it was judged expedient to send him abroad. Accordingly, in 1847, he received the appointment of Acting Naval and Military Chaplain at Bermuda, and held it for two years. That he had been from early boyhood an ardent lover of nature and a keen collector of plants and animals cannot be doubted. But it was probably during his residence in Bermuda that his future career as a naturalist took a definite beginning. Among the officers of the 42nd Highland Regiment, quartered there at the time, was Henry Maurice Drummond (brother of Drummond of Megginch), who had been stimulated into active natural history pursuits by coming under the influence of Hugh Edwin Strickland, until he made himself more than a mere amateur ornithologist. Tristram caught from him the same spirit of scientific observation, and took up the study of shells and birds in the serious way which he never afterwards abandoned. On his return to England, in 1849, he was presented to the living of Castle Eden, in Durham, and in 1850 married a daughter of P. Bowlby, an officer who had served in the Peninsular and Waterloo campaigns. Eight children were born of this marriage, consisting of one son and seven daughters.

To the duties of a country clergyman he for some years added those of tutor, and took pupils (with whom he made occasional excursions to the Continent, travelling one year along the West Coast of Norway as far as the Arctic Circle). The lung affection, however, which had necessitated his seeking the warmer climate of Bermuda, again returned upon him. He was advised to spend a winter in Algeria. From this change, which he took in the winter of 1855—56, he received so much benefit that he repeated his visit next winter, and he used to refer to these two sojourns in Africa as the prime cause of his being able to throw off the ailment which had threatened him. Having formed a friendship with the French Governor-General, he was enabled to push his explorations to the furthest French outposts, and beyond these far into the desert, living almost all the time under canvas. In the year 1857 he was joined there by Mr. W. H. Hudleston and the late Mr. Osbert Salvin. This party succeeded in making large ornithological collections, which proved to be of great interest. During the following year (1858—59) Tristram travelled widely in the Eastern Mediterranean basin, including Palestine and Egypt.

In 1860 he became Master of Greatham Hospital and Rector of Greatham, and held these appointments until 1873 when, having obtained a Canonry in Durham Cathedral, he removed to Durham, which thereafter became his home until the close of his life. But his love of travel led him to return again and again to the East in order to gather fresh material illustrative of its geology and natural history. He renewed his acquaintance with Palestine in 1863—64, and again in 1872. In 1881 he travelled through Mesopotamia and Armenia. In 1891 he visited China, Japan, and the North-West of North America. In 1894 he was again in Palestine, and once more in 1897, at the age of seventy-five. On his last visit he had his leg broken by a kick from a horse when riding near Jerusalem, but such was his irrepressible vitality that, after a few weeks in hospital, he reappeared as hale and hearty as ever.

Throughout all these extensive wanderings Tristram showed the true instincts of a born naturalist, cultivated and enlarged by wide and constant experience. To him we are mainly indebted for our knowledge of the plants and animals of Palestine and the surrounding countries. His papers on the ornithology of Northern Africa, which appeared in the 'Ibis' for 1859 and following years, were important additions to what had previously been known on the subject. His frequent journeys through Palestine allowed him to acquire an unrivalled acquaintance with the geology, topography, and natural history of that country, and he gathered together an admirable account of his observations in his great work on the 'Fauna and Flora of Palestine,' which was published by the Palestine Exploration Fund in 1884. His scientific labours and his descriptive powers, however, were made more widely known by the separate volumes which appeared from his facile pen in successive years. The first of these, 'The Great Sahara,' published in 1860, at once established his place as an accomplished traveller and observant naturalist. It was followed by a series of attractive narratives of his wanderings through Palestine.

His friend, the late Professor Alfred Newton, remarked that "Tristram's study of the 'desert forms' of the birds induced him to declare in the 'Ibis' for 1859 (p. 429) his conviction 'of the truth of the views set forth by Messrs. Darwin and Wallace in their communication to the Linnæan Society,' adding that 'it is hardly possible, I should think, to illustrate this theory better than by the larks and chats of North Africa.' Three or four pages follow in which special examples are cited in illustration, and these were written, if not published, before the appearance of 'The Origin of Species,' so that Tristram appears to have been the first zoologist to accept publicly the principles of Darwinism." "He had to modify his expressions some time after, when the 'orthodox' tide was flowing, just as Galileo was obliged to do, but he held them all the same until the end, and great credit is due to him for this."*

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* From MS. notes supplied to the writer by Professor Newton, who also, in 'Nature,' for March 16, 1906, called attention to Tristram's early Darwinian pronouncement.

"In all his voyages and journeys, ornithology received Tristram's chief attention. Among his discoveries may be especially mentioned that of a starling-like bird, named after him by Mr. Sclater, *Amydrus Tristrami*, peculiar to the gorge of the Kedron, and belonging to a genus previously thought to be purely Ethiopian. But his collection was not at all confined to specimens obtained by himself or his companions on his travels, extensive as these were; but comprehended the birds of the whole world, and formed one of the largest ever brought together by any private person. It was sold in his lifetime to the Free Public Museum of Liverpool."* It was described in the Report of the Committee of that institution for 1896 as containing "20,000 specimens, referable to 6000 species, of which 150 are types." Tristram likewise amassed a large and valuable collection of birds' eggs, which he sold to Mr. Philip Crowley, at whose death it passed by will into the Natural History Museum, South Kensington.

Canon Tristram endeared himself to a wide circle of friends by the singular modesty and geniality of his nature, by his keen sense of humour, the great range of his acquirements in natural history, and the delightful flow of his conversation, in which he would draw upon his wide and varied experience in so many different countries. He celebrated his golden wedding in the spring of the year 1901. Two years later his wife died, and he himself, retaining his faculties to the end, passed away on March 8, 1906, at the ripe age of eighty-four.

A. G.

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* MS. of Professor Newton.

ALFRED NEWTON, 1829—1907.

By the death of Professor Alfred Newton, the ranks of British zoologists have lost one of their most venerable and distinguished ornaments, and Ornithology in particular has been deprived of its most learned and accomplished British representative. Born at Geneva on June 11, 1829, he spent his boyhood with his numerous brothers and sisters at Elveden, an estate on the borders of Suffolk and Norfolk, which belonged to his father. His undergraduate life, which began at Cambridge in 1848, does not appear to have been marked by any conspicuous success in the usual subjects of study, though he is said to have gained a considerable reputation in his college for his English essays. Certainly his literary style gave proof of his having cultivated the humanities. His natural bent, however, was already strongly pronounced towards natural history pursuits, which at that time met with but little encouragement at the university, nor were his tastes favoured by his own family, as they did not seem likely to lead to any kind of successful career.

In 1853, however, after having taken his B.A. degree, he was elected at Magdalene College to the Drury Travelling Fellowship, which is open to the sons of Norfolk gentlemen. He was thus enabled to throw himself heart and soul into the active prosecution of science. He went abroad during several years, and made various journeys through Arctic latitudes, studying the abundant bird-life of these regions. Lapland, Iceland, and Spitzbergen were successively visited by him in the course of these wanderings, and, not improbably, he then imbibed that affection for northern forms which distinguished him. He likewise took occasion to cross the Atlantic more than once. In 1857 he was in the West Indies, and went thence to confer with the naturalists of the United States in Philadelphia and Washington. In 1862 he spent some time in Madeira.

During those fruitful years of active experience, his ready pen was busy in the description of the facts which he had observed at home and abroad. He communicated his notes to the pages of the 'Zoologist' and 'Ibis,' of which latter journal he was one of the original founders. For a long succession of years his numerous papers in these publications, and in the 'Proceedings of the Zoological Society,' on the occurrence, distribution, structure, and habits of birds, formed notable contributions to Ornithology. They so fully established his reputation as an experienced naturalist that in 1866 he was appointed to the newly-created Chair of Zoology and Comparative Anatomy in his own university. Afterwards his college elected him to a Foundation Fellowship. For more than thirty years, up to the time of his death, he lived in the picturesque Old Lodge of Magdalene, surrounded with his books and papers, always busy with important and useful work, delighted to welcome his friends to his den, and constantly on the outlook for opportunities of doing a kindness to younger men, especially to those who had tastes akin to his own. His Sunday evening receptions were an important feature in the scientific life of the University. Not a few of the naturalists who have risen into prominent positions in this country can look back to the stimulus they received from those meetings, where the advantages to be derived from personal intercourse among the younger workers were enhanced by the ever ready sympathy and encouragement of the genial professor.

Newton was all his life a keen collector. His chief interests lay, of course, among birds, but he had the instincts of a true naturalist, and was always on the watch for specimens in all provinces of the animal kingdom which would help to enlarge and enrich the Museum at Cambridge. He was likewise a lover of books, and his rooms, with their well-filled shelves, showed the wide range of his literary tastes, and the success with which he had pursued the quest after rare and valuable works in natural history. He was, above all, a philosophical naturalist, intent rather on the higher and broader questions than on details of species or of structure. He was endowed, too, with a highly critical faculty, and could express his criticisms with pungent clearness. He could never be satisfied with anything less than the completest accuracy attainable, while his literary instinct led him to cultivate great simplicity of style, in which every word was well chosen, and none was redundant.

These characteristic features of the Cambridge professor are specially to be noted in the numerous essays which he wrote on questions of large biological interest, such, for example, as the series of articles from his pen which appeared in the ninth edition of the 'Encyclopædia Britannica,' and which formed the basis of his greatest work, the 'Dictionary of Birds.' This ornithological classic, issued in successive parts from 1893 to 1896, was prefixed with a Latin inscription to his youngest brother, Edward, who "for more than fifty years had been his most assiduous fellow-student in ornithological pursuits at home, abroad, under the open sky, and in caves." It shows his critical acumen alike in what he selected for treatment and in what he omitted. His habitual caution is well illustrated by his choice of an alphabetical rather than a taxonomic arrangement of his subject, while the occasionally caustic force of his language is displayed in his preface, where he denounces some attempts at systematic arrangement as "among the most fallacious, and a good deal worse than those they are intended to supersede." "I have no wish," he adds, "to mislead others by an assertion of knowledge which I know no one to possess."

Alfred Newton was one of the first naturalists in this country to give in his adhesion to the views propounded by Charles Darwin as to the origin of species. A few years after the publication of these views he contributed to the 'Proceedings of the Zoological Society' (1863) an interesting confirmation and illustration of Darwin's remarks on the way in which seeds may be dispersed by birds, describing the case of a partridge which had been found with its foot firmly imbedded in a lump of hardened earth. In the address which he gave to the Department of Botany and Zoology at the meeting of the British Association in 1876, while praising the then recently published volume by Alfred Russell Wallace on "The Geographical Distribution of Animals," he emphatically refers to the modern theory of evolution as worthy of "the chief glory in giving a real and lasting value to the interpretation of the facts of animal distribution."

The subject of the distribution of plants and animals over the surface of the globe was one to which Newton devoted much thought, and on which he wrote with his characteristic breadth, caution, and critical discernment. His treatment of the "Geographical Distribution of Birds" in the ninth edition of the 'Encyclopædia Britannica' may be referred to as an excellent example of the way in which he looked at such questions of wide biological bearing. He naturally took a deep interest in everything connected with the extinction of species. In the address to the British Association above referred to, he drew a vivid and humorous picture of the effects of human interference with the economy of nature, picturing the consequences of man's occupation of an island, as seen in the destruction of its indigenous fauna and flora, and their replacement by the animals and plants introduced by him—pigs, goats, rats, rabbits, ferrets, sparrows, and starlings. He entered an eloquent plea for an endeavour to protect and preserve the native forms, and he claimed that the naturalist alone had the knowledge that should guide the efforts to promote the use and prevent the abuse of the animal world. Unfortunately, though something has since been done in the direction pointed out by him, the indiscriminate slaughter, which he so feelingly deprecated, still goes on in various parts of the world. That this subject lay near to Newton's heart was shown by his returning to it in his admirable article on "Extermination" in the 'Dictionary of Birds.'

One who contemplated with such keen regret the approaching extermination of many remarkable forms of life could not but feel a saddened interest in those which have disappeared within the times of human experience. Newton was a diligent collector of all the information that could be obtained regarding the Dodo. He wrote a number of papers on this subject, and his article on it in his 'Dictionary' may be cited as an illustration of the learning and the exhaustive treatment with which he could discuss a matter that strongly appealed to him. In the same way he devoted himself to tracing out all that could be ascertained regarding the haunts of the Great Auk or Gare Fowl, so recently exterminated. He published several papers on the subject, and one of the objects of his last yachting cruise was to visit the ledge among the Orkney Islands, where the bird had its latest British home.

For many years during the later part of his life Newton had an annual opportunity of enjoying pleasant and easy travel, and of visiting some of the most crowded haunts of bird life in these islands. His friend, Henry Evans, of Derby, also an accomplished ornithologist, gladly welcomed him on board his steam yacht and directed her course to any coast or island that the

Professor wished to see. Year after year "Alfred the Great," as Evans used playfully to call him, was received with open arms not only by his host, but by every member of the crew. And no one could look forward with keener zest to these holidays than Newton, when for some weeks he could escape from the cares of University life to the firths and sounds of the west and north of Scotland, where no letters could reach him, even if he had left an address behind him, which he was generally careful not to do. Nowhere could he be seen to be more completely in his element than on board of the "Aster." He loved the sea and its associations with such a sturdy affection that inclemencies of weather, by no means infrequent in those regions, never drew from him the least sign of impatience, or seemed in any degree to disturb his habitual cheeriness and his enjoyment of the cruise. Clad in the light-grey tweed suit which did duty on these voyages, but without top-coat or waterproof, he would sit for hours on some exposed part of the vessel, smoking innumerable pipes and watching for every variety of sea-fowl that might show itself either in the air or on the water. In the course of a few days sun, wind, rain and salt spray told on his complexion, which then assumed a ruddiness that would have astonished the inmates of Magdalene College.

The sharpness of his eyesight in the detection of birds on the wing, even when he had nearly reached the age of seventy years, was always an astonishment to his companions. And the enthusiasm with which each fresh form was greeted by him as it flew overhead became infectious to all on board. Most of the crew reappeared year after year from their winter employments to take their places in the annual cruises, and some of them became almost as cunning in bird-life as their master. In successive seasons Newton was in this way enabled to visit almost every bay and sea-loch from the Mull of Cantyre to the furthest promontory of the Shetlands.

He repeatedly anchored at St. Kilda, and had excellent opportunities of seeing there at the height of the nesting season the most marvellous and varied crowds of sea-fowl anywhere to be found among the British Islands. Nor were the voyages confined to the Scottish coast. He one year sailed round the whole of Ireland, and was thus enabled to compare the bird-haunts of the Irish cliffs with those of Scotland. Twice the yacht carried him round the Faroe Islands, and afforded him a further display of that boreal bird-life which from his young days had such charms for him.

These cruises formed an important element in Newton's life during his later years. He looked forward to them with almost boyish exuberance and delighted afterwards to recount their varied incidents. They not only provided a healthful and delightful holiday, but kept him still in close personal touch with birds, which had been the main interest and study of his life. In spite of the lameness which was understood to have been the result of an accident during infancy, he was often the first to enter the boat which had been got ready for a landing on some surf-beaten rock, or for a closer inspection of the caves and stacks at the foot of a bird-haunted precipice.

On such occasions, so self-dependent was he, he would gently repel offers of the assistance which was always at his service. It was only when the increasing feebleness of his limbs would have made such assistance indispensable that he reluctantly gave up the annual cruise.

Continuing to hold the zoological professorship for the long space of forty-one years, taking also an active part in the conduct of general business, Alfred Newton became a distinct living force in the University. To him should be ascribed no small share in fostering the rise and progress of the natural sciences towards a recognised place in the scheme of studies of Cambridge. His scientific reputation in the world outside was sustained within the walls of the University by the stimulating and suggestive form of his teaching, by his enthusiastic devotion to the development of the Museum of Zoology, and by his untiring but not obtrusive advocacy of the claims of science. But his wide and beneficent influence in Cambridge sprang also in large measure from his strongly-marked personality, wherein kindness, courtesy, and fidelity, were combined with a fearless independence, an impatient antagonism to untruthfulness in every shape and degree, and a habit of frankly and forcibly expressing his convictions.

A. G.

SIR JOHN EVANS, K.C.B. 1823—1908.

THE death of Sir John Evans has removed from the Royal Society one who for forty years has been among its most conspicuous members, who for half of that long period filled the office of Treasurer, and who from first to last has taken an active and useful part in the general business of the Society. His eminent capacity in the conduct of affairs, the unremitting devotion with which he employed that talent in the Society's interest, and the genial courtesy which marked his intercourse with the Fellows have given him a strong claim on their grateful remembrance.

He came of a stock wherein both science and literature had been cultivated. His grandfather, Lewis Evans (1755—1827), the first mathematical master in the Royal Military Academy, Woolwich, studied astronomy and was elected into the Royal Society in 1823. His father, the Rev. Dr. Arthur Benoni Evans (1781—1854) was headmaster of the Grammar School at Market Bosworth and a prolific writer, who published many poems and theological works, together with a book on 'Leicestershire Words, Phrases, and Proverbs' (1848). His maternal grandmother belonged to a Huguenot branch of an old French family, and from her he perhaps inherited his lightness of heart. He was born on 17th November, 1823, at Britwell Court, Burnham, Buckinghamshire, and was educated under his father at Market Bosworth. Although entered for matriculation at Brasenose College, Oxford, he did not eventually proceed to the University. His education, however, under the paternal roof had been excellent. He had acquired such a knowledge of Latin and such an acquaintance with classic authors as remained a life-long possession to him. Every now and then, in the course of conversation, some happy phrase or line from a Roman poet would occur to him, with which he would light up and enforce the remarks he was making. His archæological writings indicate how diligently he sought in ancient literature such references as might illustrate the early history of mankind. His father's care in his upbringing was further shown by his being sent for a short time to Germany, in order to gain some facility in speaking the language.

Instead of entering the University, he, in 1840, at the age of seventeen, embarked on a commercial career. His maternal uncle, John Dickinson, the head and founder of the well-known firm of paper-makers of that name, and a man of scientific tastes, who became a Fellow of the Royal Society, invited him to join the staff at the paper-making works of Nash Mills, near Hemel Hempstead. John Evans found there the settled home in which he lived almost up to the end of his life and which became more widely known as the abode of the active and enthusiastic antiquary, numismatist, and geologist than as the headquarters of a commercial company. Having married his cousin, the daughter of the head of the firm, he was, in 1851, admitted as one of the partners, and in course of time he in turn became the senior member of the firm. To those who met him only in his business relations he was an active and enlightened paper-maker, keenly alive to every modern improvement in machinery and in the processes of manufacture, gifted with great clearness of judgment and remarkable capacity for mastering the most complicated details of business. His energy and initiative largely contributed to the success of the various enterprises of the firm. For many years he was President of the Paper Makers' Association, and took a leading part in the conduct of its affairs.

But while thus sedulously attentive to commerce he found leisure to gratify his strong bent towards the study and collection of antiquities and the prosecution of several branches of scientific enquiry. His taste for geology seems to have been developed even in boyhood, for he is said, when nine years old, to have hammered a collection of fossils out of the Wenlock limestone quarries at Dudley. But his geological proclivities were eventually drawn in two main directions, partly by the requirements of his business and partly by his love of antiquities. In paper-making an ample water supply is essential, and in Hertfordshire the subject of water-rights has long been keenly discussed. Evans, in the interest of his firm, studied the question of water-supply, both from the geological and the meteorological side, and he became on these matters a recognised authority, whose advice was often sought and always valued. No one stood up more stoutly and successfully than he for the conservation of the water-supply of his county, which was again and again threatened by the great metropolitan water companies. This important question being thus forced on his attention by pressing practical considerations, he devoted much time to its study. He explored the superficial deposits in all parts of his district as well as the water-bearing strata that lie deeper underground. In the course of these enquiries he was led to investigate the relations between rainfall and evaporation, and the percolation of rain through soil—subjects regarding which little information was available at the time when he began his researches. From the year 1853 he had under his own immediate care the rain-gauges and percolation-gauges which had been erected at Nash Mills in 1836 by his uncle.

He was drawn into the geological field by another and different pathway. In the first decade of the latter half of last century the discovery of what were alleged to be implements of human fabrication in the old river gravels of the north of France gave rise to a keen discussion among men of science. The conclusion, which some of the early observers drew from the evidence, that man must have lived on the earth for a far longer period than had generally been supposed, naturally aroused much interest among the general public. As far back as 1841 Boucher de Perthes had obtained from the old gravel terraces of the valley of the Somme, at Abbeville, numerous chipped flints which he recognised to be the handiwork of man. In 1847 he began to publish his observations, but they met with little or no support among his fellow countrymen. On the contrary, they were either ignored or denied and even derided, though one or two competent French geologists were convinced of their probable truth. It was not until the autumn of 1858 that Hugh Falconer, who then saw the collection made by Boucher de Perthes at Abbeville and was satisfied that the shaped flints were truly human implements, urged Joseph Prestwich to undertake an examination of the geology of the valley of the Somme, with the view of determining the precise position of these implements and of ascertaining whether or not there was evidence to prove their high antiquity. This task was accomplished in the spring of 1859 by Prestwich, who took Evans with him to assist in the investigation. The conjoint labours of these two observers, which completely demonstrated the accuracy of the French discoverer's observations and conclusions, formed the first important step in winning general acceptance to the opinion, which had been so stoutly contested, that the human race, together with various tribes of animals that have been long extinct, must have inhabited Western Europe for a long succession of ages, wherein the rivers cut their way deeply into the valleys which they traverse. Prestwich communicated his results to the Royal Society, while Evans submitted a statement on the subject to the Society of Antiquaries, which had elected him one of its number in 1852. This paper appeared in the 'Archæologia' (vol. 38, 1860, p. 280), under the title of "Flint Implements in the Drift; being an Account of their Discovery on the Continent and in England."

The journey with Prestwich formed the turning-point in Sir John's scientific career. From that time onwards he specially devoted himself to the investigation of the earliest traces of man which have been preserved in river-gravels, brick-earths, cavern deposits, or elsewhere. He became one of the most enthusiastic and successful collectors of flint implements. His singularly good powers of observation enabled him to detect them even on ground that had been already searched for them, and in any company of hunters for these objects he was generally the most fortunate. Even on a surface so long inhabited as that of Egypt his trained eyes enabled him to pick them up. Both abroad and at home he purchased freely every illustrative type which he could procure, until in the end he had amassed such a series of these objects as is probably possessed by no other private collector.

Throughout his life he continued to publish from time to time notices of the progress of discovery in regard to the occurrence and distribution of flint implements. So recently as December, 1907, he communicated to the Geological Society what proved to be his last paper, on "Some Recent Discoveries of Flint Implements," wherein he expressed his matured opinions regarding the probable origin of the high-level gravels in which these relics of primitive man have been found.

But besides writing these scattered papers, Evans rendered a great service to the progress of archæology by his published volumes, in which he gathered together all the evidence which had been accumulating in different countries as to the types and distribution of the various relics of early human workmanship. The first of these separate works appeared in the summer of

1872 with the title of 'The Ancient Stone Implements, Weapons, and Ornaments of Great Britain.' It at once took its place as the chief authority on the subjects of which it treats. The learning displayed in its earlier chapters, the careful arrangement of its material, its detailed yet interesting descriptions, and the importance attached throughout its pages to the stratigraphical position in which the relics had been found showed it to be no mere antiquarian enquiry but a treatise conceived and executed on thoroughly scientific lines. It had an important influence in connecting the pursuits of archæology and geology, by the way in which it marshalled the evidence for a chronological sequence in the relics of early man, and showed that the conclusions derived from a consideration of varieties in types of workmanship were supported by the geological evidence derivable from the positions in which these several types were found. In the midst of the numerous and multifarious duties which claimed his constant attention he brought out a second edition of the work in 1897, greatly revised, and incorporating a large amount of new material.

Although Sir John Evans chiefly occupied himself with the archæological side of geology, he occasionally ventured into other parts of the geological domain, and his incursions of this kind were always marked by the same quickness of insight and shrewdness of inference. It was he, for instance, who first detected that the toothed jaw which lay detached on the same slab of stone that contained the original specimen of *Archæopteryx* probably belonged to that ancient type of bird—a surmise which was completely confirmed twenty years later by the discovery of a second specimen wherein the jaws with pointed teeth lay in place in the skull. At another time he devised an ingenious piece of mechanism to illustrate how he supposed that great changes of climate might be brought about without any shifting of the earth's axis of rotation. By means of a moving wheel, on the rim of which he placed a weight between the pole and the equator, he showed that the centrifugal force gradually drew the weight towards the equator and he contended that on the supposition that the interior of our globe is a liquid mass enclosed within a shell of fairly uniform density and thickness, the effect of the elevation of a great mountain chain midway between the pole and the equator would be to draw the shell over the liquid nucleus until the original position of the pole might be moved as much as 45° to the south (' Roy. Soc. Proc.,' vol. 15, 1867, p. 46). He subsequently formulated his hypothesis as a definite mathematical problem (' Quart. Journ. Geol. Soc.,' vol. 32, 1876, p. 62). The mathematicians, however, who investigated it did not regard it as tenable, and there were formidable objections to his postulate as to the condition of the earth's interior. But Evans, though he bowed to the weight of authority, probably never wholly abandoned his view.

His more purely antiquarian work hardly comes within the purview of the Royal Society, but no notice of his life would be complete without some reference to that side of his activity. He began early in life to collect and study coins, and he became in the end one of the most accomplished numismatists of his day. His first independent volume, 'The Coins of the Ancient Britons,' published in 1864, possessed singular interest and value from the abundant evidence it supplied of the existence of a gold coinage in England before the coming of the Romans, and from its ingenious proofs (which had been first published by him as far back as April, 1848), that these British coins had originally been imitations of a stater of Philip of Macedon, but by successive copying of the imitation had become so rude that, but for the preservation of the intervening stages of debasement, the origin of their pattern would never have been surmised. Another of Evans' antiquarian writings which has taken its place as the standard treatise on the subject of which it treats is his 'Ancient Bronze Implements, Weapons and Ornaments of Great Britain and Ireland.' From the nature of these objects and the positions in which they have generally been found they do not furnish the same kind of geological evidence as to their relative dates, and the author discussed them mainly from the antiquarian side. As an instance of Sir John's watchful zeal and singular success as a collector of antiquities, the writer of this notice may allude to an incident which occurred a few years ago. On his way to Greece, Evans had picked up at a dealer's in Paris a well-preserved gold coin of one of the Roman Emperors and showed it to the friends whom he met in Rome. A few days after his arrival in the Italian capital he astonished these friends by producing another beautiful gold coin which he had bought from a dealer there—a coin of the wife of the same Emperor.

Sir John Evans was elected into the Royal Society in 1864. In the course of three years he was chosen to serve on the Council, a position which he again filled from 1873 to 1875. His business capacity on the Council was further recognised in 1878 when he was elected Treasurer of the Society. This distinguished and responsible office he continued to hold for the long term of twenty years. During that period he was unremitting in his care of the Society's finances, which he left in an orderly and sound condition.* At the same time he took an active part in the conduct of the general business, his practical knowledge of affairs and his experienced judgment always giving to his counsel an especial value. On the occasion, in 1884, when the President, Professor Huxley, was disabled by ill-health, Evans prepared and delivered the Anniversary Address. As, in the absence of the President, the Treasurer, who is usually also a Vice-President, takes the Chair, Sir John had frequent opportunities of presiding both at the Council and in the meetings of the Society. His conduct as Chairman was often singularly felicitous in the tact and humour of his remarks.

While his activity in the Royal Society was thus so marked, he had time and energy to spare for the claims of other societies. He had an especial affection for the Geological Society, which, as far back as 1857, had enrolled him in its ranks. For eight years, from 1866 onward, he was one of its secretaries until, in 1874, he was chosen to be its President. In 1880 he received its Lyell Medal "in recognition of his distinguished services to geological science, especially in the department of Post-Tertiary Geology." For the last thirteen years he has been its Foreign Secretary—an office which he only resigned last February, when he found that his increasing feebleness of health prevented him from regular attendance at the meetings of the Council. At the Society of Antiquaries, the Royal Numismatic Society, the Anthropological Institute, the British Association, and many other societies he has held the highest offices, and has for many years been a familiar and valued associate.

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* On his retirement from the Treasurership, Sir John gave an interesting account of the state of the Society's finances, and showed how considerably the funds had increased during the time in which he had held office.—'Year Book' for 1899, p. 160.

Those who met him only at scientific meetings in London might naturally take him to be a denizen of the capital, entirely engrossed in the work of the various societies in which he played so prominent a part. In reality, his headquarters were always at his home, in Hertfordshire. Not only was he fully immersed in the conduct of the paper-making works at Nash Mills, but at the same time for many years he stood out as the most active and prominent public man in the county. He was appointed High Sheriff of Hertfordshire in 1881, and for some years he filled the offices of Chairman of Quarter Sessions and Chairman of the County Council. The friends and neighbours who chose him for these responsible positions, whether or not they could appreciate his reputation in the scientific world outside, knew him at home as a worthy county gentleman, more capable than most of them of grasping and directing business matters. The universal testimony of the authorities in Hertfordshire at the time of his death was a touching tribute to the influence which he exerted among them, to their high personal esteem for him, and to the great value of the services which he had, in many varied ways, rendered to his county. It was a fitting recognition of these great public services, as well as of his reputation as an antiquary and man of science, when his neighbour, Lord Salisbury, in 1892, asked Queen Victoria to confer on him the honour of K.C.B.

Sir John Evans was married three times. His first wife, to whom allusion has already been made, left three sons and two daughters. One of these sons is the well-known explorer of Knossos, and now a Fellow of the Royal Society. The second wife, daughter of Mr. Joseph Phelps, died without children. Lady Evans, who survives her husband, is the daughter of Mr. Charles C. Lathbury, Wimbledon, and an accomplished classical scholar and antiquary. She has one daughter.

Those who were privileged to know Sir John Evans in the intimacy of private life mourn the loss of a true friend and a charming companion. His advice, so often asked and so freely and cordially given, has been a guide to many who survive him, for his long experience of men and things gave to his judgment a clearness and decision which were eminently helpful. He would spare himself no effort actively to serve one in whom he took interest. His invariable courtesy of manner seemed to belong rather to the quiet stateliness of a past generation than to the hurried intercourse of modern life. In a difficult situation, where tact as well as firmness was required, his qualities were altogether admirable. His conversation, always interesting, was often witty. He could rapidly throw off impromptu verses in which some passing incident was humorously depicted, and his memory, stored from a wide range of reading, enabled him often to interject a happy quotation. These characteristic features he retained almost unimpaired up to the last, even though the ailment which finally carried him off was gradually sapping his strength and causing him much suffering. He bore this burden bravely to the end, and died on 31st May, 1908, in the 85th year of his age.

A. G.

HENRY CLIFTON SORBY, 1826—1908.

THE ranks of British geologists have lost one of their most distinguished ornaments by the death of Dr. H. C. Sorby, who for more than half a century has been looked up to all over the world as the great master by whom modern Petrography has been regenerated. He came of a family that has been connected with the staple industry of Sheffield since the sixteenth century. One of his ancestors was the first Master Cutler of the Cutlers' Company, who died in 1628. His grandfather became, in turn, Master Cutler. His father was a partner in the well-known firm of John and Henry Sorby, edge-tool manufacturers. His mother, Amelia Lambert, of Queen's Square, London, appears to have been a somewhat remarkable woman, from whom he not improbably derived most of his versatile ability and powers of concentration.

He was born on May 10, 1826, at Woodbourne, near Sheffield, an estate which belonged to his father and which he inherited. His early education was obtained at the Sheffield Collegiate School. He used to tell that he there obtained, as a prize for arithmetic, a book entitled 'Readings in Science,' to which he ascribed no small influence in giving him his bent towards research and experiment. His tastes in that direction were further fostered, after he left school, by a mathematical tutor, the Rev. Walter Mitchell, who, having had a medical training, had become a fairly good anatomist and chemist, and who initiated his pupil into these subjects, besides superintending his mathematical studies. When this accomplished teacher left him, young Sorby, not being under the necessity of choosing a profession, determined to devote himself to a scientific life. He continued to study mathematics, optics, chemistry, and anatomy. He found time also for the prosecution of water-colour drawing. "I worked," he said, "not to pass an examination, but to qualify myself for a career of original investigation."* It must be acknowledged that this training was eminently successful in producing a man of science who, gifted with remarkable originality of mind, marvellous industry, unwearied perseverance, and singular mechanical ingenuity, attained distinction in various branches of science, and left his mark on every domain of research into which he entered.

Being in possession of ample means, he determined to remain at Sheffield, where opportunities for conducting experimental research offered themselves, and he made that town his home up to the end of his long life. He resided with his mother until her death in 1874. Thereafter, being free to take longer journeys, he bought a yacht, the "Glimpse," and for many years spent the summer months dredging and making biological and physical observations in the estuaries and inland waters of the east of England. The winters were spent in Sheffield, carrying on his experiments. For some years past he is known to have been engaged in working up the results of various researches made long ago, of which the details had never been published. Even when confined to bed, in the last months, from the effects of an accident, his mental activity continued as vigorous as ever. During that time he prepared and sent to the Geological Society a long and elaborate paper, and carried on his correspondence with his own hand. He continued to busy himself with his notes even up to the day before his death. On the night of Sunday, March 9, last, he lost consciousness, and lingered till the following evening, when he quietly passed away in the eighty-second year of his age.

In looking over Sorby's published papers, more than one hundred and fifty in number, one is first impressed by the extraordinary mental versatility which they display, and the uninterrupted continuity with which they came from his pen, from the time when he was one-and-twenty, up to within a few days of his death. His earliest published communication appears to have been one "On the Amount of Sulphur and Phosphorus in Various Agricultural Crops," which not only appeared in the 'Memoirs of the Chemical Society' and the 'Philosophical Magazine,' but was translated into 'Froriep's Notizen.' It was probably suggested by some of the chemical studies which he had carried on with his tutor. He soon struck out into a more original path. It was into the geological domain that, influenced by his environment, he was first attracted, and it was there that he found the widest field for his peculiar powers, and achieved his greatest success. The rivers Don and Rother, which flowed near his home, drew his attention by the evidence presented in their valleys that the streams had not always kept to their present channels, but had wandered to and fro across their alluvial plains. He was thus led to examine the internal arrangement of alluvial deposits, and by the happy accident of a rain shower, which led him to shelter in a sandstone quarry, his eye caught the profile of the lines of current-bedding in the old Carboniferous alluvia. He at once perceived the interest and importance of these lines as indications of the direction and variation of the currents by which the sediment had been transported and laid down.

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* The writer of this obituary notice is indebted for information to an Address by Dr. Sorby on his 'Scientific Investigations during the last Fifty Years,' given to the Sheffield Literary and Philosophical Society, on February 2, 1897, and published in the 75th Annual Report of the Society in 1898. The occasional citations in this notice are taken from that Address.

For more than ten years Sorby continued to devote much time to the study of this subject. He watched the action of streams and of sea-waves in the deposition of detritus, and contrived an ingenious piece of mechanism by which this action could be illustrated. Travelling over the country, he was always on the watch for rock-sections in which the history of sedimentation could be traced. By the evidence which these sections afforded he determined the direction of the currents that had carried the various layers of sand and gravel, and he speculated upon the probable site of the land from which this detritus had been derived. In this way he attempted to reconstruct the geography of the country at different geological periods. Thus the internal structures of the Oolitic rocks of the Yorkshire coast suggested to him the former existence of land between Norway and Scotland. Another series of observations furnished him with materials for discussing the probable physical geography of Central Scotland during the time of the Old Red Sandstone. He wrote a succession of papers in which he applied the same methods to the investigation of the former condition of the south and south-east of England during different times of geological history.

In these early researches, as we now learn, there were included many elaborate experiments, the detailed results of which were not published at the time. These results and a full discussion of their bearing on the history of sedimentation occupied the time of their author during the last months of his life. They were communicated by him to the Geological Society only a few weeks before his death, and they appeared after that sad event in the form of the paper already referred to, consisting of more than sixty pages with five plates, which has since been published in the 'Quarterly Journal' of the Society. This interesting memoir, his last legacy to the science he loved so well, supplies an excellent illustration of the manner in which he sought to apply experimental physics to the study of rocks. It is full of suggestion, and even where the results he obtained may seem too uncertain to warrant implicit confidence in them, they may serve to show the lines along which further research should be prosecuted. He was himself perfectly aware that some of them were probably only approximately correct, but he felt justified in giving them to the world. "It appears desirable," he said, "to do the best I can with the material at my disposal, hoping to lead others to do what I intended to do, and correct such errors as are now unavoidable."* The seed which he thus sowed may yet yield a further harvest of knowledge in this department of geological investigation.

At an early stage in his career Sorby vividly realised the fascination and the potency of the microscope as an instrument of scientific research. His youthful studies in optics had, no doubt, made him familiar with all the details of its mechanism, and capable of adapting it to any line of investigation in which he might find it of service. It was natural that he should first apply this instrument to the solution of some of the problems which his work among sedimentary rocks suggested. As far back as the year 1831 a method had been devised and described by William Nicol of Edinburgh, whereby slices of fossil-wood could be mounted on glass and made so thin as to become transparent. In this form these sections were shown to reveal every detail of their internal organisation. Nearly twenty years passed before any geologist seems to have been induced to avail himself of this means of studying the minute structures of rocks. The first who did so was Sorby. He began by examining thin sections of the Calcareous Grit of the Yorkshire Coast, and he sent a communication on this subject to the Geological Society, which appeared in the 'Quarterly Journal' for 1851. As he advanced in his investigation of the microscopic characters of limestones and marls, he saw that the same method of study might be applied not only to sedimentary rocks, but to those of igneous origin, including even the most fine-grained and opaque. He thus entered a wholly new and untrodden field in geological enquiry.

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* 'Quart. Journ. Geol. Soc.,' vol. 64, p. 172 (May, 1908).

The chemical composition of igneous rocks had long been investigated and was fairly well known. But though the component minerals of close-grained masses might be more or less probably surmised from the results of chemical analysis, no really accurate and precise information on this subject could thereby be obtained. In the early years of last century, indeed, Cordier had shown that by crushing fine-grained rocks and washing and separating the grains of their powder, their component minerals might be isolated and examined under the microscope. But this method threw little or no light on the relations of these minerals to each other in the genesis of the rocks composed of them. In 1858, however, Sorby's great paper "On the Microscopical Structure of Crystals indicating the origin of Minerals and Rocks" was published by the Geological Society. This masterly essay revealed that much of the origin and history of igneous rocks could be ascertained by means of the microscope. Not only was it shown to be now possible and easy to determine the several minerals, even in a close-grained rock, but to discover the order in which they had successively crystallised, the conditions of temperature and pressure in which their solidification had taken place, and the alterations which the rock composed of them had subsequently undergone. Sorby, besides making use of transmitted and reflected light, was familiar with the delicate applications of polarised light, and found with what great advantage they could be employed in the study of rocks.

It was from this memorable paper that the remarkable modern development of the petrographical side of geology took its rise. In every country where the study of rocks is pursued, the methods first indicated by Sorby have been followed. While many developments and improvements of his methods have been introduced, he is everywhere acknowledged to have been the "Father of Modern Petrography." To have entirely revolutionised this important branch of research and to have opened a new and boundless field of investigation into the past history of our globe will ever constitute his chief claim to a high place among his scientific contemporaries.

One of the geological problems which as far back as 1851 had interested Sorby was that of the origin of the slaty cleavage of rocks. This subject had been discussed by various observers, notably by Adam Sedgwick, who worked out with great skill the distribution of the chief lines of cleavage that have affected the rocks of Wales. He showed the intimate relation between the strike and the cleavage of large disturbed masses, and thus prepared the way for the true solution of the problem, although he himself favoured the notion that the structure was the result of the action of electric currents. Daniel Sharpe subsequently insisted that cleavage must be due to mechanical pressure, but as Sorby remarks, "little notice was taken of what he said, because he did not show that the ultimate structure of the rock was really such as would be produced by this cause." This relation of the effect to its producing cause was first experimentally demonstrated by Sorby. He satisfied himself that cleavage has no connection with electric currents, but is simply due to great mechanical pressure, whereby this structure has been superinduced in rocks along planes perpendicular to the direction of the pressure. He found the microscopic structure of cleaved rocks entirely to support this view, which he further illustrated and confirmed by ingenious experiments. Thus, by mixing scales of oxide of iron with pipeclay and subjecting the mass to strong lateral pressure he obtained a perfect cleavage structure. In his paper descriptive of these observations, published in 1853, he dwelt on the proofs which, as thus interpreted, the cleavage structure furnishes of the gigantic compression undergone by mountain masses during their elevation. His contention was soon afterwards supported by Tyndall and others, who showed that even in homogeneous substances like beeswax a cleaved structure could be induced by mechanical pressure. Hence, for more than half a century cleavage has now been recognised as one of the most convincing proofs of the enormous compression which the more disturbed parts of the earth's crust have undergone. For the establishment of this fact geologists are, in the first place, indebted to Sorby.

In the course of his investigation of the minute structures of rocks, and with the accumulating evidence before him of the enormous pressure to which many parts of the earth's crust have been subjected, it occurred to him that the mechanical force involved in great subterranean pressure may have been partly resolved into chemical action, as in other circumstances it might be resolved into heat, electricity, or other modification of force, and that in this way various puzzling appearances in rocks might receive an explanation. Accordingly, in his usual way, he set to work to test this hypothesis by direct experiment. After making a large series of investigations, he succeeded in proving that there undoubtedly is a direct correlation between mechanical pressure and certain kinds of chemical action. The results of this important research formed the subject of the Bakerian lecture which he gave to the Royal Society in 1863. The most striking geological illustration of the truth of his conclusion was furnished by him from various limestone conglomerates which have suffered severe compression and in which the pebbles have indented each other, the solution of their substance being greatest at the points of contact where the effects of pressure were most pronounced.

Sorby's interest in chemical questions, which began in his youthful days with his tutor, continued active all through his life, and gave a special character to many of his geological and mineralogical papers. One of his early observations, for example, related to the origin of magnesian limestone by the alteration of an ordinary calcareous deposit. In another enquiry, which involved an extensive series of experiments, on the production of artificial pseudomorphs, by the action of cold or highly-heated solutions, he showed that certain rocks, like the Cleveland Ironstone, were originally composed of carbonate of lime, which has been replaced by carbonate of iron derived from the associated strata. Again, he conducted a long investigation into the occurrence of the two forms of carbonate of lime—calcite and aragonite—in the shells of mollusca, and he was thereby led to some interesting and important conclusions. He found that some shells are composed of calcite, some of aragonite, and others partly of the one and partly of the other in distinct layers. He ascertained, further, that calcite, being in a state of stable equilibrium, could not be altered into aragonite; whereas aragonite, being unstable, could easily pass into calcite. Hence calcite shells may be preserved in a limestone and even retain their microscopic structure, while those made wholly or partly of aragonite may have lost their internal structure or may have been entirely effaced.

His microscopic studies of minerals had usually a chemical side. This feature of his work was especially illustrated by the numerous papers which he wrote in the year 1869. He then announced some new applications of the microscope to blow-pipe chemistry. He detected and described the minute crystals which he had detected in blow-pipe beads. He carried out an elaborate investigation into the nature of the liquids observable in various minerals, especially in sapphires, rubies, spinels, aquamarines and emeralds, and made many measurements of the rate of expansion of these liquids with increase of temperature. In the fluid cavities of sapphires he found that the volume of the liquid, when heated from 0° to 30° C., expands from 100 to 150, and he, consequently, inferred that it must be liquid carbonic acid. In the course of these researches, he had occasion to study the zircon, or so-called "jargon," of Ceylon, a mineral in which a remarkable assemblage of distinct elements had been shown by spectrum analysis to be present. At first he believed that some peculiar and characteristic spectra, which he obtained in the jargons, indicated the presence of a new element, to which he gave the name of Jargonium. Further examination, however, convinced him that this conclusion was erroneous, and that the peculiar spectra belonged to some compounds of the oxides of uranium with zirconia. With characteristic frankness he at once published an acknowledgment of the mistake.*

From the investigation of the microscopic structure of terrestrial rocks Sorby was naturally led to enquire into the structure and probable history of those masses of mineral matter which come to us from outer space in the shape of meteorites. He soon found that the olivine enclosed in these stones contains excellent "glass-cavities," proving that it was once in a state of igneous fusion, likewise "gas-cavities," like those so common in volcanic minerals and indicative of the presence of some gas or vapour. He ascertained that the minerals in meteorites, usually considered to be identical with those in terrestrial volcanic rocks, nevertheless present some characteristic differences in structure. When he turned to the siderites and siderolites or iron-meteorites, he soon saw that in order to gain an insight into their structure and probable origin it was desirable first to study various artificial irons. In this research he ascertained that certain microscopic structures very closely similar to those in some varieties of meteorites, could be artificially produced. He was thus enabled to indicate, as far back as 1864, how much may be learnt as to the structure and composition of different types of artificial iron by the aid of the microscope. Notwithstanding the obvious practical importance of his observations and conclusions in relation to the development of our iron industry, they attracted no attention. At last, after some twenty years, the matter was taken up seriously in 1887 by the Iron and Steel Institute. Sorby was then requested by that Society to consider, together with Dr. John Percy and Sir Henry Bessemer, the best way of illustrating a complete paper on the subject. "In those early days," he remarks, "if a railway accident had occurred, and I had suggested that the Company should take up a rail and have it examined with a microscope, I should have been looked upon as a fit man to send to an asylum. But that is what is now being done. What I really proved was that various kinds of iron and steel are varying mixtures of well-defined substances, and that their structure is in many respects analogous to that of igneous rock I also took specimens of iron and steel and acted upon them with acid, so that it was possible to print from them as from types, and show many interesting points connected with their structure." Sorby continued his investigation and published various papers on the subject during the following decade. He is now recognised as the great pioneer in micrometallography, and his methods have proved of great practical use in the manufacture and testing of iron and steel. As a mark of this recognition one of the most important constituents of steel has been named after him, Sorbite.

One of Sorby's most useful inventions to which he was led in 1865 by his study of meteorites is the spectrum-microscope.† He applied this instrument to a large number of different substances, and published some forty papers in different branches of scientific enquiry wherein colour plays a part, such as the pigments in human hair, birds' feathers, the shells of birds' eggs, and the colouring matter in almost every group of plants. One of the practical applications of this invention to which its author attached importance was the detection of blood-stains. He stated that "as small a quantity as a hundredth part of a grain may be detected under circumstances in which it would be utterly impossible to recognise it either microscopically or chemically even if present in much larger amount." It is interesting to note that many long years after these researches were made, when he lay on what proved to be his death-bed, he returned to this subject and collected some of his notes on experiments upon the colouring matters of plants, which he sent in the form of a communication to 'Nature'—the last paper which he published.*

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* 'Roy. Soc. Proc.,' vol. 17, p. 511, and vol. 18, p. 197.

† It was fully described, together with its method of use and its application, in 'Roy. Soc. Proc.,' vol. 15 (1867), pp. 433–455.

After the year 1879 Sorby spent five months of every year on board his yacht chiefly among the waters that surround and penetrate the low coast line of the south and east of England. Having no rocks to notice in this region, he was led to enter new fields of enquiry wherein he manifested the same mental activity and ingenious mechanical resourcefulness. Meteorological changes engaged much of his attention. He wrote on the colour of the clouds, sky, and sea, and on forecasts of the weather as deduced from the rainfall and changes in the barometer. For many years he continued to take observations of the temperature of the estuaries and more open waters. In 1882 he spent seven hours a day for 240 days in studying the Thames in connection with the enquiries made by the Royal Commission on the Drainage of London. He applied the microscope to the detection of sewage-contamination and of the purifying influence of minute animals and plants. His numerous researches on these and other subjects enabled him to lay before the Commission a large mass of important evidence which he believed had considerable influence on the findings in their Report.

At frequent intervals he published accounts of the scientific results of his yachting cruises. These were usually communicated to the Sheffield Literary and Philosophical Society, sometimes to the 'Essex Naturalist.' More detailed observations on some of the groups of animals he collected at sea were now and then sent to the Linnæan Society. But perhaps the most interesting and memorable outcome of these cruises was the ingenious methods which he devised and perfected for preserving even the most perishable forms of marine life and exhibiting them as permanent preparations or as effective lantern-slides. At the soirées of the Royal Society in 1898 and 1899, he exhibited some of these preparations which attracted much attention from the perfection with which the internal structure of the animals was revealed by them. He had found by experiment that a great variety of modes of treatment for different animals was required to secure the best results. Excellent transparent lantern-slides of some forms were obtained by mounting the objects on glass with Canada balsam. Actiniæ and other organisms were killed with menthol, the addition of a little of which to sea-water was found to cause the animals to expand very fully. In this distended condition they were preserved in formalin. It was further ascertained that excellent preparations of various marine animals could be made by placing them in strong glycerine, the index of refraction of which is so nearly that of the soft tissues of these organisms, that it makes them more or less transparent; they then look bright and life-like, and much of their internal structure is distinctly visible.

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* 'Nature,' January 16, 1908, p. 260.

It has to some of Sorby's friends been matter of regret that he did not himself follow up the consequences of his own discoveries, but left this to be done by others, while he himself passed on into fresh fields of observation and experiment. But when his peculiar gifts are considered, it may be felt that he not improbably employed them better in the course which he adopted. He seemed to flit from subject to subject, but there was generally a well-connected mental chain in these transitions. When new ideas were suggested by the progress of one of his enquiries, he was apt to branch off into collateral investigations, which might soon become in his eyes more fascinating and important than that out of which they arose. He used to say of himself that his difficulty was to avoid discovering new things, but that for him, at least, it was "possibly better to invent new things than to work up old ones thoroughly." It must be admitted that there were few subjects to which he for a time devoted serious attention, which he did not enlarge and illumine.

This habit of divergence necessarily led to the accumulation of a vast mass of notes of the results of observations and experiments. In 1898, when receiving the presentation of his portrait, he said of himself: "The majority of my friends can have little idea of the amount of material I have collected in connection with a variety of subjects. The great difficulty I now have is to find time to work that material into shape and to publish it. I hope to be able to do so, but I am beginning to think it is a doubtful question."

How Sorby could be, as it were, enticed into one enquiry after another, until he had travelled far away from his starting-point, is well illustrated by his own account of what followed his prolonged investigation of the Thames. While engaged in studying the estuary of that river, he was naturally interested in the remarkable topographical changes which the surroundings of the Isle of Thanet have undergone within historic times. The evidence of these transformations, partly geological and partly antiquarian, suggested to him various enquiries, in the course of which he found it necessary to examine Roman, Saxon, and Norman buildings, to study their respective building materials and to carry out a great many experiments. In seeking further light on the subject from illuminated manuscripts he was struck by the variations in the unit of length employed by the scribes, and he drew some inferences therefrom as to the several countries where the MSS. had been written. Once embarked on the study of manuscripts he could not resist the temptation to enquire into ancient conceptions of cosmogony and geography, the archæology of natural history, and the origin of the ideas connected with the more or less mythological animals met with in ancient art. In pursuance of the quest he took up the study of Egyptian hieroglyphics, since he found so many of the natural history stories to be traceable back to Egypt and Babylonia. Addressing his friends in Sheffield on these matters he added, "with a view of carrying out this very extensive subject, I have collected most of the original works of importance from the earliest period down to mediæval times. This study will, I hope, ultimately lead to some important results in connection with the history of science and art." His vast preparations, however, remain behind him as a memorial of the indefatigable energy, the vivid interest in a wide range of enquiry, and the thoroughness of method which he retained unimpaired at the age of more than three score years and ten.

Dr. Sorby was elected a Fellow of the Royal Society in 1857. In 1863 he gave the Bakerian Lecture to which reference has above been made. He received a Royal Medal in 1874 "for his researches in Slaty Cleavage, and on the minute structure of Minerals and Rocks; for the construction of the Micro-spectroscope, and for his researches on Colouring Matters." He served on the Council in 1876—77.

His connection with the Geological Society was not less close. He became a Fellow of that body in 1850, and in 1869 received the Wollaston Medal, the highest distinction in the gift of the Society. He was elected President in 1878, and during the two years in which he held office he gave two memorable Addresses in which he discussed the minute structure and the origin of limestones and of non-calcareous sedimentary rocks. He communicated a number of masterly papers to the Society's Quarterly Journal, the last of the series, as already mentioned, having been his latest scientific achievement, written in bed shortly before his death.

Living all his life at Sheffield among his books and experiments, he was personally known to a comparative small circle of his contemporaries. Those who had the advantage of his acquaintance or his friendship were struck with his unassuming, childlike, trustful disposition, his kindly and helpful ways, and, above all, with the singularly absorbing ardour with which he would talk about what was engaging his attention. He would discourse with the same animation, and almost in the same language, to a child as to a master of a subject. One of his peculiarities was a scrupulous regard for his health. It is supposed that he never in the course of his life got thoroughly drenched with rain.

In his native town, where his fellow citizens were well aware of his high scientific reputation, his participation in municipal affairs would have been heartily welcomed. But he always shrank from mingling in public life. His name, indeed, was added to the Commission of the Peace, but he was hardly ever seen on the magisterial bench. He was keenly interested, however, in educational matters, especially in the provision of adequate technical instruction. Thus he was one of the active founders of the Firth College, out of which the Sheffield University has sprung, and the benefactions made by him during his life to that institution have been supplemented by a generous legacy in his will. It was in the rooms of the Literary and Philosophical Society, however, that he appears always to have found the most congenial company. For more than half a century he was the most active and useful member of that body. He was always pleased to communicate to it accounts of the progress or results of his researches, whether carried on in the laboratory or on board of the yacht. The jubilee of his connection with the Society was fitly celebrated in November 1898, by the presentation to him of his portrait as a token of personal esteem and a recognition of his world-wide fame as a man of science. This portrait, which now hangs on the Society's walls, has been well reproduced in autotype. It is an excellent likeness, which will serve to perpetuate the features of one of the most distinguished of the geologists of the Victorian era.

A. G.

SIR JOSEPH FAYRER, 1824—1907.

Sir Joseph Fayrer was born at Plymouth on December 6, 1824, and died at Falmouth on May 21, 1907. His father was a naval officer who served under Lord Cochrane, Marryat the novelist being one of his messmates. On his mother's side he was descended from John Copeland who took David, King of Scots, prisoner at the battle of Neville's Cross. His childhood was spent in the Lake District where he knew Wordsworth, Hartley Coleridge and John Wilson (better known as Christopher North), the editor of 'Blackwood's Magazine.' At the age of fifteen he began to study engineering, but he was very anxious to go to sea, and, as he was too old for the Navy, he made several voyages on a merchant vessel. On one of these he visited Bermuda during an epidemic of yellow fever, and became so much impressed with the importance of the medical profession that he determined to enter the medical service of the Navy, and accordingly commenced his studies at Charing Cross Hospital in 1844. One of his fellow students, with whom he contracted a warm friendship, was Thomas Henry Huxley, and this friendship, to a great extent, determined Huxley's career. In the chapter of autobiography prefixed to his Essays, Huxley says: "I was talking to a fellow-student (the present eminent physician, Sir Joseph Fayrer) and wondering what I should do to meet the imperative necessity of earning my own bread, when my friend suggested that I should write to Sir William Burnett, at that time Director-

General for the Medical Service of the Navy, for an appointment." This appointment he obtained, went for his famous cruise on the "Rattlesnake," and made the zoological observations which not only brought him fame, but settled his path in life.

After finishing his medical studies and becoming qualified to practise, Fayrer obtained a commission in the Navy, and was sent to the naval hospital at Haslar, where one of the assistant-surgeons was Andrew Clark, afterwards President of the Royal College of Physicians. Fayrer had only been a short time at the hospital when Lord Mount-Edgcumbe invited him to travel with him. They travelled together through Germany, Switzerland, and Italy. While they were at Palermo, fighting occurred between the Sicilians and Neapolitans. At Rome, he took his degree of M.D., being the first Protestant on whom it had been conferred. After his travels were over he did not return to the Navy, but obtained a commission in the Artillery and sailed for India on June 29, 1850, before his friend Huxley had returned from his voyage on the "Rattlesnake." In less than two years he was sent to Burmah, where he so distinguished himself that Lord Dalhousie appointed him to the post of Residency Surgeon at Lucknow, regarding which he says in an autograph letter: "I have purposely reserved it, that I might bestow it, as the best medical appointment in the gift of the Governor-General, upon the assistant-surgeon who should be found to have rendered the most approved services during the war with Burmah."

The extraordinary energy which had gained Fayrer distinction found full scope in the manifold duties of his new office, for, in addition to his work as Residency Surgeon, he had to superintend the hospital and other institutions, and to fill the office of postmaster. Shortly afterwards, he was appointed honorary Assistant-Resident, so that he was obliged to add political work and correspondence to his already onerous duties. When the King of Oude was deposed, the care of his horses, elephants, camels, wild animals, and artillery was thrown, in addition, upon Fayrer's shoulders. In spite of it all, he managed to reorganize the Post Office, and to extend its operations over the whole province. When the Indian Mutiny broke out, Fayrer's house at Lucknow was used both as a fortress and hospital, and during the famous siege he not only did his share of fighting, but had to prevent sickness from overcrowding, and to treat the wounded, among whom were Henry Lawrence, Outram, and Napier.

After the Mutiny was over, Fayrer returned to England, broken down in health, in March, 1858, but, instead of resting, as most men would have done, in order to recuperate, he entered at Edinburgh University as a medical student, rubbed up his classics so as to pass the preliminary examination, studied chemistry, botany, and anatomy, worked at the hospital, passed a special examination, and took his doctor's degree in March, 1859. A month later, in April, 1859, he began work as Professor of Surgery in the Medical College Hospital of Calcutta. Here, again, his wonderful energy enabled him to do the work of several ordinary men. Besides his lectures at the hospital, he had to give courses of operative surgery, perform numerous operations, and attend to private practice. In spite of all this, however, he managed to find time for scientific work, and made investigations into the pathology of various febrile and septic diseases of India which had previously received there little or no attention. He took up the hygiene of hospitals, and drew official attention to the defects in structure and sanitation which rendered the Indian hospitals unhealthy. But the research in which he took the greatest interest was his zoological work on the snakes of India, and his physiological investigation into the action of their venom. The difficulties under which his scientific work was carried on are shown by the fact that he had often to leave an experiment in order to attend to his hospital work, and while there amputating a limb, or performing some other operation, his mind would be disturbed by anxiety regarding the condition of his private patients who were anxiously waiting for him.

His scientific interests were very wide in character. It was in consequence of meteorological work that he had done that he was elected a Fellow of the Royal Society of Edinburgh in 1859. When on the Council of the Asiatic Society of Bengal, he proposed an ethnological investigation of the races of India. This proposal produced some useful reports, but was never fully carried out. Another proposal to form a Zoological Society and Gardens in Calcutta, which he made when President of the Asiatic Society in 1869, was more fortunate, and, though delayed for a time, it was ultimately carried into effect.

For a time Fayrer was surgeon to the Governor-General and also President of the Medical Faculty of the University. In 1870, he accompanied the Duke of Edinburgh on his travels through the North-West of India, and Lord Mayo in the Terai in the following year. In 1872, his health failed, and he returned to England, where he became President of the Medical Board at the India Office. His magnificent work on the Thanatophidia of India had been published by the Government, and, after his return to England, he resumed, in collaboration with Lauder Brunton, the researches he had begun in India on snake venom. Their researches on Cobra Venom were published in the ‘Roy. Soc. Proc.’ No. 145, 1873, and No. 149, 1874, and on Crotalus Venom in ‘Roy. Soc. Proc.’ No. 159, 1875. They examined the antidotal action of many substances, and found that permanganate of potash, which Fayrer had already tried, appeared to be most promising (‘Roy. Soc. Proc.,’ 1878, vol. 27, p. 465).

In 1875, Fayrer accompanied King Edward VII, who was then Prince of Wales, on his tour through India, and but for his extensive knowledge and firm decision in difficult circumstances, the Prince might have been induced by the earnest entreaties of various personages to visit infected places, with the probable result that cholera might have spread over large districts of India, and that our King might never have returned from his visit to that part of the Empire.

In 1876, he was elected a Fellow of the Royal Society, and was a Member of Council in 1895. As President of the Medical Board at the India Office, he had much to do with matters of public health, and in addition to his official work, he became President of the Epidemiological Society, in 1879, gave the Croonian Lectures on the "Climate and Fevers of India," at the Royal College of Physicians, in 1882, represented the Government of India at the International Cholera Conference, in Rome, in 1884, and was President of the Section of Preventive Medicine in the Hygienic Congress in London, in 1891.

He was a good linguist, and was obliged to acquire a fair knowledge of Hindostani and Persian, in order to conduct the correspondence necessitated by the offices he held at Lucknow. He knew sufficient Italian to be qualified to pass the examinations for M.D. at Rome, and to make a speech in Italian when he was representing the Royal College of Physicians at the Tercentenary of Galileo at Padua.

The law regarding experiments on animals prevented him and Brunton from continuing the researches on antidotes to snake venom they were making in 1875, but in 1903 Captain Leonard Rogers was able to continue their work by means of Professor A. D. Waller's method of keeping animals continuously under chloroform for thirty-six hours or more. In conjunction with him they published a joint paper in the 'Roy. Soc. Proc.,' 1904, vol. 73, p. 323, and the method there described has since been successful in saving several lives which would otherwise have been lost.

In trying to sum up Fayrer's work, one meets with the great difficulty that so much of it was official, and the credit for such work goes rather to the office than to the individual. Thus the enormous amount of good which he did in his official capacity cannot be estimated, except from the official recognition it received, not only during the Burmese War, but in every office which he filled.

His chief scientific work consisted in his early meteorological observations, his proposal of an ethnological investigation of the races of India, his foundation of a Zoological Society and Zoological Gardens at Calcutta, his contributions to sanitation and to the pathology of Indian diseases, and, most of all, in his work on venomous snakes. His monumental work on the Thanatophidia of India is the best and most comprehensive on the subject, and the researches which he began in India, and continued with the collaboration of others in England, have now led to a method of treating the bites of venomous snakes, which can be applied to bites of all kinds, and used everywhere.

There was a remarkable similarity, in many respects, between Fayrer and his friend and fellow-student Huxley, and this likeness was the attraction which drew them together, and led to their close friendship. It has been said that in every human face a resemblance may be traced to some animal, and this was markedly the case both in Fayrer and Huxley. Especially in his later years, Huxley's face and head suggested that of a lion, while Fayrer's large open forehead and calm expression reminded one of an elephant, and one could hardly look at him without thinking how rightly the Hindoos have chosen an elephant's head for their god of wisdom. Both men were alike in the extraordinary energy they possessed, in the stern uprightness of their characters, in the extent of their knowledge, and the wideness of their interests, in the clearness of their views, the correctness of their decisions, their absolute fearlessness, their prompt and energetic action, their firm determination to carry out whatever they thought right, in their tenacity of purpose, in a certain impatience of opposition, and in their great success in overcoming it. Associated with these qualities which compelled admiration were an extraordinary kindness and tenderness of heart, which gained the affection of all who knew them. In Fayrer, the writer of the Notice lost one of his best and truest friends, who could always be confidently relied upon in case of need. This feeling was shared by every one of Fayrer's friends, from the lowest to the highest. At his funeral, one of the wreaths bore the gracious inscription: "For auld lang syne, from Edward VII."

L. B.

[The Obituary Notice of Lord Kelvin has been issued as No. A 543 of 'Proceedings.']

SIR MICHAEL FOSTER, 1836—1907.

Born at Huntingdon, March 8, 1836, eldest son of Michael Foster, F.R.C.S., of that town, he was educated at Huntingdon Grammar School until he was thirteen years of age, when he proceeded to University College School, in 1849.

Here, under the tuition of Dr. Key, the head master, he distinguished himself in classics, and in 1854 took his B.A. degree at the London University, on the Arts side, taking the first place and the University scholarship in classics. So great was his bent towards classics, and so highly did Dr. Key think of his abilities, that he would have undoubtedly tried for a classical scholarship at Cambridge, had the fellowships been at that time open to Nonconformists ; but the Foster family is distinguished among Non-conformists in East Anglia. To this early classical training, and to his great friendship with Huxley, that master of lucid scientific writing, is undoubtedly due the wonderfully clear and fascinating style of all his writings.

At University College School he took great interest in cricket, and during his time there was captain of the eleven. His love of the game remained throughout his life. During his residence at "The Granhams," when his students were few in number, he inaugurated an annual cricket match beween the staff of the laboratory and the students, in which he always took part. This annual match was played on a field belonging to him, and was a great success; subsequently, when the class became too large and the physiological laboratory was but one of many others, this match became replaced by one between the teachers in the various laboratories and the assistants. Foster was captain of one side, and played in the match regularly up to 1895.

Cambridge and a classical career being closed to him, it was determined that he should follow his father's footsteps and enter the medical profession. Accordingly, in 1854, he entered the medical side of University College, London, and the practice of the hospital.

In 1856, he obtained gold medals in anatomy and physiology and in chemistry, and took his M.B. degree at London University in 1858 proceeding to the M.D. degree in the following year.

In the year 1859–1860 he went to Paris to continue his medical studies, and returned to England in 1860. At this time, signs of pulmonary disease appeared, and he therefore obtained an appointment as surgeon on the S.S. "Union," which went to the Red Sea to build a lighthouse on the Asaruf Rock, opposite Mount Sinai.

Throughout his life, or at all events through the earlier part of his career at Cambridge, the dread of consumption haunted him, and from the time of his first arrival, he made up his mind that he could not live in Cambridge itself, but that as far as possible he must live an open air life. For this reason, he took a house—"The Granhams"—at the foot of the chalk hills known as the Gogmagogs, nearly four miles from Cambridge, and later, in 1879, he built for himself a house on the summit of one of these hills on the same road as was his former house. In both houses he tried to make a rule of spending the afternoon in his garden, often working hard with the spade or pick. Excellent as this plan proved in staving off further tubercular troubles, it certainly prevented him from obtaining that full weight in the councils of the University to which his ability and the services he had rendered the University entitled him, for the morning hours are taken up in teaching and the administrative business of the University is of necessity undertaken in the afternoon.

In 1861, he commenced practice with his father at Huntingdon, and remained there until 1867, having, in 1863, married Miss Georgina Edmonds, daughter of Mr. Cyrus Edmonds. His married life with his first wife was short, for she died in 1869, leaving him two young children to look after.

For six years he remained in practice, but all the time his longing was for a scientific career. He had joined the British Association in 1859, and had attended the meetings in Oxford, Cambridge, and Dundee, so that when an invitation came from his old teacher, Sharpey, to give a course of practical physiology at University College, he accepted it, and relinquished medical practice for ever.

Sharpey's influence over Foster was very great; in after years he used always to say that in the dark ages of physiology in England, when physiology was a mere appendage to human anatomy, the only teaching being in most schools a course of lectures given by the professor of human anatomy and physiology, it was Sharpey alone who kept the lamp of research alight, he alone who recognised that advance in physiological knowledge could come only by experimentation, he alone who instilled into the minds of all his pupils that lectures by themselves were of little use, but that the student must see experiments for himself in order to obtain a real knowledge of the subject.

Besides Sharpey, Foster was much influenced by Claude Bernard, although when he was in Paris he does not appear to have attended any of his lectures. His appreciation and admiration for him appear in every page of that delightful memoir of Claude Bernard written by him for the 'Masters of Medicine Series,' a book, the dedication of which runs as follows:—"To the physiologists of France, both to those who had the happiness to know Claude Bernard in the flesh and to those who, like myself, never saw his face, this little sketch is dedicated in the hope that, as he has been to me a father in our common science, so may I be allowed to look upon them as brethren." A third great influence in the making of Foster was undoubtedly Huxley. He succeeded Huxley as Fullerian Professor of Physiology at the Royal Institution in 1869, and in 1870, when Huxley commenced his course of elementary biology at South Kensington, he was assisted by Foster, Ray Lankester, and Rutherford. Just as Sharpey and Claude Bernard had impressed upon his youthful mind the importance of experimental work in the study and teaching of physiology, so his intercourse and friendship with Huxley led him to take the broadest views of the meaning of physiology, strengthened his biological bias, and was, perhaps, the chief cause of his life-long effort to place physiology at the head of the group of biological sciences.

On the Continent at this time, experimental research in physiology was much more universal in the laboratories than in England, but there, too, the instruction was mainly given by lectures, with demonstrations during the lectures; there was no organised system of practical instruction.

The practical course of physiology and histology, inaugurated by Sharpey, which he gave over entirely into the hands of Foster, retaining for himself the theoretical part of the teaching, was unique in Great Britain at that time, and to it must be traced the emancipation of physiology from the bonds of human anatomy. The time had come when physiology was to take its true place in the annals of science and the experimental method, the only true way of advancing scientific knowledge, to attain by leaps and bounds its present high position in Great Britain; the man alone was required, and that man was Foster.

As is so frequently the case in scientific advance, the initiative came from the University of Cambridge, not indeed from the University itself, for that is a body ill provided with funds and slow to act, but from Trinity College. The original suggestion came apparently from George Eliot and George Henry Lewes, who were great friends of W. G. Clark. He and Coutts Trotter felt, and persuaded the College, that the time had come when it would be of advantage to the University for separate teaching in Physiology to be given. They therefore approached Huxley and asked him to help them; he replied without hesitation, "I know the very man for you, a young fellow at University College called Foster."

So Foster came to Cambridge as Trinity Prælector of Physiology, not belonging to the University but in it. As far as the University was concerned he had no status, no vote in the Senate, for though an Hon. M.A. degree was conferred upon him in 1871, it did not in those days carry with it any of the privileges of the ordinary M.A. degree. He was ineligible for election to any Board of Studies, could therefore only make his voice felt in the University through his friends.

The University granted one small room, now part of the Philosophical Library, to the Trinity Prælector of Physiology. Here Foster began his lectures, and here was at first his only laboratory for histology, chemical and experimental physiology. He brought with him, from London, H. Newell Martin as his demonstrator.

From this small beginning arose the whole Biological School of Cambridge.

Foster's teaching was a revelation: it was all new, not to be found in any English text-book, all so suggestive, opening out vistas of research, showing how little was known, how much remained to be found out.

Up to that time Humphry, who was Professor of Anatomy and Physiology, had given an annual course of physiology, interesting undoubtedly, but based on structure rather than on experiments on animals; the only practical work a few mounted specimens shown under a microscope. In those days there was no separate examination in physiology for the second M.B. examination. No wonder that Foster's lectures came as a revelation, and, in combination with the enthusiasm and sympathy of the man, caused many of the small band of his earliest students to decide there and then to take up a scientific career and follow him.

At the time when he came to Cambridge, biological sciences were represented by professors of the old school. Humphry was Professor of both Anatomy and Physiology, as was customary in medical schools of that time, and was well known as a surgeon and anatomist. Babington was Professor of Botany; his lectures consisted largely of the botany of the flowering plants, and he was distinguished as a systematist. Newton was Professor of Comparative Anatomy and Biology, and his attention was concentrated on the anatomy and distribution of the vertebrates; he was particularly distinguished for his knowledge of birds.

Foster, who, with Huxley, had initiated the teaching of elementary biology on evolutionary principles, and was thoroughly imbued with the great principle that physiology was one of the biological sciences, and must go hand in hand with botany and zoology, from the very first determined to form a biological school in Cambridge, which should be of the most advanced character and second to none.

For this purpose he carefully studied the bent of his various students, and picked out Balfour to study the new science of embryology and Vines to work at the new botany. In his own department of physiology he had besides H. N. Martin as workers and helpers Langley, Lea, Gaskell, and Dew Smith. By this means he gradually built up a school of biology of a newer type, running side by side with the University teaching, unpaid by the University, recognised only by the allocation of rooms. Here was Foster's strong point: rooms there must be for practical work and research. He would have nothing to do with the old system of teaching almost entirely by lectures. From the first the new zoology and the new botany must have rooms for practical work just as much as the new physiology.

Such a growth was only possible, owing to the endowments of the colleges; for these young and enthusiastic teachers who gave their whole time to Foster and his work, with neither appointment nor salary from the University, could not have done so but for the fellowship system and the recognition by their respective colleges that they were doing good work worthy of support.

This, however, would have availed but little but for the man himself. Not only would he point out the direction in which advance in any science was to be looked for, but by his earnestness, his lovable charm of persuasion, his entire freedom from any thought of monetary gain, or any kind of selfishness, the conviction was gradually borne in on his pupils that the particular line of research on which each was engaged was the one thing in life worth doing, and that the only place to do it was in Cambridge by Foster's side. As Foster used to say, the true man of science must feel with respect to his own research that "in this way only lies salvation." It was that feeling that he had so pre-eminently the power of raising in a man.

Soon the fame of the Cambridge Biological School began to spread over the country, and a very rapid increase in the number of medical and scientific students took place. The makeshift buildings in which Foster, Balfour, and Vines had up to this time taught their students were hopelessly inadequate, and new laboratories were a crying need. With the help of his friends, especially Coutts Trotter, Balfour, H. Sidgwick, J. W. Clark, and Newton, the University was persuaded to build a biological laboratory, which was completed in 1878. Subsequently this laboratory was extended so as to give more room for physiology and zoology, and quite recently a splendid botanical laboratory has been erected.

In 1883, consequent on the Report of the Royal Commission, a professorship of physiology was founded, and Foster was elected to the chair; the complete degree of M.A. was conferred upon him, and at last, after 13 years, he was able to speak for himself in the Administrative Boards of the University.

In 1872 he married Margaret, the daughter of Mr. Rust, Cromwell House, Huntingdon, who survives him.

Foster held very strong views as to the proper method of teaching physiology to students at the beginning of their medical study. He held it to be a mistake to demonstrate during the lecture, and insisted that practical work, carried on by the student himself, illustrative of the facts on which the lecture was based, must immediately follow the lecture. The physiology of each organ must be dealt with as a whole in lecture, and the practical work must be so arranged as to bring home to the student all the points of each lecture at the time, and not to be regardless of the lecture, as must be the case if the practical work is departmental while the lecture course is general. His ideal laboratory would be of sufficient size to provide every student with his own working place, both in the histological and in the chemical department at the same time. He also—and this was one of the great reasons of his success—encouraged his pupils at the very earliest moment to engage in some original research, and then persuaded them to give a few lectures of an advanced character upon the subject on which they were working; for, as he said, there is no way of discovering the gaps in your knowledge of a subject better than lecturing on it. In this way he associated with himself a band of younger workers engaged in research, who gave the advanced teaching to the students, thus allowing him to confine himself to the introductory course.

In the researches suggested to the students and in the minds of his students themselves, he always inculcated the close connection between the physiology of all living organisms, insisting upon physiology being a branch of zoology and of botany, to be studied as the greatest scientific biological subject, and no longer to be looked upon as an adjunct to anthropotomy.

With all these broad-minded philosophical ideas, he would never have succeeded to the extent that he did but for his personal influence not only in his own circle but in the University at large, which enabled him to establish respect for a new study in University circles naturally wedded to older studies. In about fifteen years, his and his group's influence succeeded in obtaining University and College recognition in fairly full measure for the subject as one on a par with the older studies of the place.

It is impossible to overestimate Foster's services to physiology. He was the prime mover in bringing together all English workers in physiology by the foundation of the Physiological Society. At its origin the Society was formed in consequence of the passing of the Vivisection Act in 1876, and was simply for the purpose of dining together at stated times with the object of interchanging views and keeping watch on the working of the Act; subsequently it was determined that a scientific meeting should precede the dinner, and Foster insisted from the very first that such meetings should, as far as possible, be demonstrational.

This gathering together of English physiologists into a society has been of enormous benefit to the progress of physiology. Still greater, perhaps, was the foundation of the 'Journal of Physiology.' Up to 1878 the only journal, apart from the 'Proceedings of the Royal Society,' in which physiological papers were published, was the 'Journal of Anatomy and Physiology,' edited by Humphry and Turner. The time had come, in Foster's and in Sanderson's opinion, for a journal devoted exclusively to physiology, and he determined to found one. From the very first the journal was run on dignified lines—paper, typography, figures, and plates being so good as constantly to excite admiration in the United States, in Germany, and other places—a policy which certainly for a long period of time involved financial loss.

The foundation of the 'Journal of Physiology' is yet another instance of the devotion which Foster inspired in his pupils, for it was the admiration which Dew Smith had for him which led him to insist that what Foster undertook should be of the best kind, regardless of expense; Dew Smith undertook to bear the financial loss, and he took the Journal under his special care as far as plates, illustrations, paper, and printing went. His affection for Foster, and his desire in every way to further his interests, led to the founding of yet another most valuable aid not only to physiology but to science in England generally, viz., the Cambridge Scientific Instrument Company. At that time laboratory research was much hampered in England by the difficulty of getting any special instrument made for any particular research. In Germany the instrument makers were willing to make special instruments for which there was not likely to be any further sale, but in England it was very much more difficult. The large instrument makers looked upon it as a special favour to undertake such an order, and often the research was delayed because of the length of time required in making some necessary piece of apparatus. Dew Smith saw that here he could greatly help Foster and physiology, and so he started in Cambridge a workshop expressly to turn out anything required in scientific laboratories as quickly as possible, to which was afterwards added a drawing and lithographic establishment. This workshop, the foundation of which was indirectly due to Foster, has been a great boon to science, especially in its later development as the Cambridge Scientific Instrument Company.

Foster held very strongly the opinion that science was cosmopolitan, that all workers in physiology should be banded together in one brotherhood—the brotherhood of science; the practical carrying out of this belief manifested itself in two ways, both of which have been very fruitful and of great benefit to English physiologists. In the first place, when he started the 'Journal of Physiology,' he determined to obtain American as well as English co-operation, and on the title-page of the first number appear the names of Bowditch, Martin, and Wood, as well as of Gamgee, Rutherford, and Sanderson.

In the second place, when the International Medical Congress met in London in 1881 he and Kronecker together drew up a scheme for a separate International Congress of Physiologists to meet every three years, and a Committee was formed. The first Congress was held at Basel in 1889 and they have been held with great success every three years since that date. Their success is largely attributable to Foster's powerful influence, always exercised and repeatedly emphasised to make the proceedings demonstrational, to the exclusion of papers and to make the social proceedings as cheap and informal as possible.

In 1872 he was elected a fellow and in 1881 he succeeded Huxley as one of the secretaries of the Royal Society, a post which he held until he resigned it in 1903. During these twenty-two years Foster's influence in the world of science was very great indeed; he became personally acquainted with the leading men of science of every department, and with his broad-minded scientific spirit he set himself to further and aid scientific progress in every direction; thus apart from purely biological subjects he took an active part in the establishment of the National Physical Laboratory, in the rearrangement of the Meteorological Office, and in starting the International Congress of Geodesy. In 1897 he was president of the physiological section of the British Association at Toronto and in 1899 he was President of the Association at the Dover meeting.

Perhaps his most important work as Secretary was the establishing of close, confidential, and frequent relations between the Royal Society and Government Departments. Foster believed this to be to the advantage both of the country and of the Society; many of the Society disagreed with him strongly on this point; whether right or wrong in his judgment he carried that policy through until the Society had become expert adviser to a number of Government departments as a routine thing. These departments placed great confidence in Foster and in numberless cases he stood with them for the Royal Society itself. The way in which the Government departments went at once in medical matters to the Royal Society for advice and assistance, as if it were the fountain of medical knowledge as well as scientific, simply represented the great reliance placed on Foster.

All who knew him, especially those who worked with him at the Royal Society during these twenty-two years, felt how intensely Foster cared for and devoted himself to the welfare of the Society, inspiring all of those with whom he worked with a conception of the Society as a living active factor in the life of the nation, as well as the leading scientific club.

He was a member of the Committee appointed by the Colonial Office to advise as to the best means of preventing malaria and other tropical diseases, and his services were recognised on his death by an official letter from the Colonial Office to Lady Foster.

He was appointed by the Government to serve on various Royal Commissions: that on vaccination in 1889, on the disposal of sewage in 1898, and on tuberculosis in 1901, of which latter he was Chairman. For these services and in recognition of his services to the cause of science he was created in 1899 Knight Commander of the Order of the Bath.

Foster, who was a great reader of scientific papers in all languages, always impressed upon himself and others the maxim that the next best thing to knowing a thing is to know where to find it; and he felt strongly that unless steps were taken soon it would become very rapidly more and more impossible to know where to find it. He had already been one of the most active promoters of the Royal Society Catalogue of Scientific Papers, and he threw himself heart and soul into the greater scheme of an International Catalogue of Scientific Papers. He was the soul of the whole thing: he got the money, he raised the enthusiasm, and by the Royal Society starting it and insisting that English is the proper language for it because bound to be the most nearly universal, a stride was made in the right direction and work done that would be more difficult and costly to do later than then.

The undertaking is not yet certain of success but undoubtedly it is one of the utmost importance to the scientific men of all countries, and its very magnitude bears testimony to the boldness and persuasiveness of its author.

Nor were his energies expended only in enterprises directly connected with science. He never forgot his close connection with University College and the University of London, and he was an active member of the Statutory Commission by which the University was reorganised, and teaching functions added to the examining powers which it previously exercised.

Foster's actual additions to our knowledge by way of research are small and not of great importance. He was a discoverer of men rather than of facts, of biologists rather than of facts and theories in biology. He was an impulsive man, he very rapidly decided and acted, especially quickly did he come to a conclusion about a man's character and ability. The judgment of a man once thus rapidly formed he never seemed to relinquish. His judgment in the great majority of cases was curiously correct; in some cases it was wrong and in these it always seemed as though Foster thought that the man himself had changed not that his first impression was incorrect.

With respect to his scientific work, his paper "on the Action of the Constant Current upon the Heart of the Snail," in conjunction with Dew Smith, is a model of the way in which a research should be thought out and a scientific paper written. He it was who was one of the first to study embryology in this country, and he used to tell how he believed he was the first man to cut up an embryo chick, and mount every section in the right order in series throughout. Huxley, he said, was very pleased, and had thought such a feat hardly possible.

It was a memorable day in the history of biology when Foster, talking in the little room of the philosophical library about his future career with Balfour, who wanted to devote himself to science, but was uncertain what line of research to follow, took up an egg, cracked it, showed him the embryo inside, and said "What do you think of working at that?"

Foster's influence extended far beyond the limits of his own country. His 'Text-book of Physiology,' published in 1877, made him known throughout the world. In the excellency of its literary style, and the suggestiveness of its criticism of the unsettled problems of physiology, it was far superior to any other text-book, in recognition of which it was translated into Italian, German, and Russian. In America it was *the* text-book. In 1876, through Foster's influence, H. N. Martin became Professor of Physiology at Johns Hopkins University, Baltimore; he took over with him his enthusiasm for Foster and for his methods of teaching, and, with the aid of the text-book, which appeared in the following year, he revolutionised physiological teaching in the United States. To this day Martin is looked upon in the States as the father of the present-day method of teaching.

Apart from his text-book, Foster was known and loved throughout the physiological world. When he was elected perpetual Honorary President of the International Congress of Physiologists in 1901, there was a great prolonged outburst of applause, that seemed as though it would never stop. At the Congress of 1904, at Brussels, when he was not well enough to be present, he sent a telegram: "Though absent, I am with you." Immediately the International Committee interrupted the business going on in order that the President might read the telegram. There was genuine distress at his ill-health among the people present

This appreciation of Foster throughout the world was manifested by the various distinctions bestowed upon him. He received honorary degrees from the Universities of Dublin, Glasgow, Montreal, Oxford, and St. Andrews, and was appointed honorary or corresponding member of a large number of learned societies both at home and abroad. In 1900 he was urged by many of his friends to stand for the representation of London University in Parliament, and finally, after a good deal of hesitation, accepted and was elected. He had always belonged to the Liberal party; he belonged to a family of strong nonconformist views, but could not side with Gladstone on his views about Home Rule. He was therefore nominally a supporter of the Government, and spoke and voted on that side. His speeches, mainly on scientific matters, were always received with great attention, and his influence in the House was considerable. When, however, the Education Bill of the Government was brought forward, he found himself so strongly opposed to their views on the religious question that he felt he could no longer sit on that side of the House, especially as, in addition, he had no sympathy with any suggestion of Tariff Reform. Instead of resigning, he crossed the floor of the House, urging that he was elected as an Independent Member to represent the University of London, that he was known by all to be a Liberal in politics, not a Conservative, and had been sent to Parliament to supply a crying want—the representation of science.

His opponents made the most of his non-resignation, with the result that in 1906, when he was again a candidate, he was defeated, though by the narrow majority of twenty-four votes.

In many ways his entrance into Parliament was unfortunate. The impossibility of attending to his Parliamentary duties without neglecting his professional ones, or *vice versâ*, combined with the fact that he represented London, not Cambridge University, brought about his resignation of his professorship, in 1903.

In the same year he resigned the Secretaryship of the Royal Society, so that in 1906, when he was not re-elected to represent London University, he found himself stranded. Still, however, he was by no means devoid of occupation, for the Commission on Tuberculosis, of which he was Chairman, and the Sewage Commission, provided work and interest.

It is a matter of great regret that Cambridge University possesses no Professorial Pension Fund. If ever there was a man in the University whose services should have been recognised by a retiring pension, Foster was the man.

His great delight outside his scientific work was horticulture, and it was ever a source of wonder to his many friends that he was so successful in making rare plants grow on a bare chalk hill-side. His special fancy for a long time was the growing of irises, of which he had a wonderful collection ; he was especially successful with the Onocyclus group, of which he manufactured many new hybrids. His patience was remarkable, in many cases the seeds did not germinate till the fifth year, and in some not till the eighth year or even later from the time of planting. In later years he grew especially, in addition to irises, Eremurus of different kinds with great success.

His knowledge of horticulture and his delight in plants was recognised in the botanical world by his appointment as Chairman of the Departmental Committee, to report on the botanical collections at Kew, and at the British Museum. He wrote many articles, especially in connection with irises, for the horticultural journals.

One of the new studies which he was particularly keen to introduce into Cambridge was the scientific study of agriculture. It is mainly due to his energetic advocacy that Cambridge to-day possesses a flourishing agricultural school, and it was a great source of delight to him when the University determined to follow his lead and provide for the teaching of agriculture on a scientific and practical basis.

Zoology shared with botany his active interest; it was due largely to his co-operation with Ray Lankester, and his active support, that the Marine Biological Research Laboratory at Plymouth came into existence; an instrument of research which, with other kindred establishments, has proved of the utmost value to the progress of zoology.

His power of grasping and presenting to others a mass of complicated detail was remarkable. As senior Secretary he had for many years to bring the business to be dealt with before the Council of the Royal Society, and through his skilful presentations of the facts, the Meetings ended with but few arrears. He held his own views tenaciously, but accepted an adverse vote with good humour. He was keenly alive to the desirability of keeping the Council in close touch with the Society, and it was largely owing to him that the existing custom of the presentation of an annual report by the Council to the Fellows was introduced.

Foster was a delightful companion, full of humour, with an irresistible bubbling over kind of laugh, and a twinkle in his eye which was most infectious. He was excellent as an after-dinner speaker, and was usually expected to speak; frequently, some unexpected humorous but never ill-natured allusion to some distinguished person at the dinner, would cause roars of laughter; almost as frequently the laugh would be directed against himself.

Up to the very last he was at work, up to the last he retained his gift of humorous speaking, and on the very day on which he died (January 28, 1907), he had made an excellent speech in London at the Meeting of the British Science Guild.

This last public appearance was a fitting end to a great career. There have been many greater scientific men than Foster. It is hardly too much to say that no man ever devoted himself more whole-heartedly to science, and that if science can be served by strengthening the influence and promoting the spread of the scientific spirit, few have done it better service.

W. H. G.

PROCEEDINGS OF

THE ROYAL SOCIETY.

Section B.—Biological Sciences.

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*Further Results of the Experimental Treatment of Trypanosomiasis in Rats; being a Progress Report of a Committee of the Royal Society.**

By H. G. Plimmer, F.L.S., and J. D. Thomson, M.B., C.M.

(Communicated by Sir Ray Lankester, K.C.B., F.R.S., Chairman of the Tropical Diseases Committee. Received October 28,—Read November 7, 1907.)

[Plate 1.]

The following results are a continuation of the work already described, and the experiments have been carried out upon rats inoculated with the same strains of Nagana and Surra:—

Condition of the Animals living at the Date of the Completion of the Tables in the former Paper.

Table I.—Nagana Rats treated with Atoxyl and Succinimide of Mercury.

No. 4 is still living and well 229 days after inoculation.
„ 7 „ „ 222 „ „
„ 10 „ „ 178 „ „
„ 15 „ „ 164 „ „
„ 21 „ „ 63 „ „
„ 6 died on the 214th day after inoculation.
„ 12 „ 110th „
„ 16 „ 81st „

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* This Committee, which planned and supervised the investigations, was appointed by the Tropical Diseases Committee for the purposes of this experimental enquiry, and consists of the following members: Professor J. Rose Bradford, Colonel Bruce, Professor Cushny, and Mr. H. G. Plimmer. A preliminary summary of the results of the enquiry

In these rats the livers were found to be pale and fatty, and the kidneys degenerated: these were pale and fatty, with fibrous streaks, and the urine of Nos. 12 and 16, found in the bladder *post mortem*, contained albumen. They did not die of the disease, as no trace of trypanosomes could be found in either of them, but of the degenerative changes mentioned.

Table II.—Surra rats treated with Atoxyl and Succinimide of Mercury.

No.	5	died on the	206th	day after inoculation,	of pneumonia.
„	9	„	169th	„	„ „
„	14	„	61st	„	from an unnoticed recurrence.
„	15	„	59th	„	from paralysis after three recurrences.
„	18	„	61st	„	.
„	19	„	59th	„	.
„	20	„	57th	„	after five recurrences, becoming finally axotyl-proof.

In Nos. 5 and 9 there was also evidence of fatty and fibrous degeneration of the kidneys.

Of these rats only in Nos. 14 and 20 was there any evidence that they died of the disease.

Table III.—Rats treated with Atoxyl and Mercury Sozoiodol.

No. 5 is still living and well, 208 days after inoculation.
„ 3 died on the 181st day after inoculation.

(The kidneys and liver of No. 3 were very fatty.)

Table V.—Rats treated with Atoxyl and Donovan's Solution.

No. 2 died on the 70th day after inoculation.
„ 6 „ 121st „ „

(The kidneys of No. 2 were degenerated, those of No. 6 markedly so, being pale in colour with yellow streaks, and very friable; there was albumen in the urine.)

Table VI.—Rats treated with Atoxyl and Iodipin.

No. 9 is still living and well 218 days after inoculation.
„ 10 died on the 141st day after inoculation.
„ 14 „ 178th „

(No. 14 had also 1·2 milligrammes of succinimide of mercury. The kidneys of this rat were degenerated, and it had albumen in its urine.)

From the above list it will be seen that the principal pathological lesion in those rats which have been treated with atoxyl and some compound of mercury and have lived for a very long time after inoculation, being, we think, cured of the disease, is a degeneration of the kidneys; and in most of

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has already appeared in the 'Proceedings of the Royal Society' (B, vol. 79, 1907, pp. 505—516). By the courtesy of the governing body of the Lister Institute the investigations have been carried on in the laboratories of that institution.

these rats this was the only lesion found *post mortem.* This will be referred to later in mentioning further treatment with mercury.

Atoxyl and Calomel.

Twelve rats have been treated with atoxyl (three to five doses) and then with subcutaneous and intramuscular injections of calomel, in doses of 1 minim of Surgeon-Major Lambkin's formula. It is difficult in rats to make the injection into the muscles, and in all cases necrosis occurred at the site of the injection; no better result was attained than by treatment with atoxyl alone, with subsequent recurrences and death.

Atoxyl and Succinimide of Mercury.

Further experiments have been made with this combination, in which the dose of the mercury salt has been increased up to 1 milligramme. The 12 rats of this series are all dead, and showed acute kidney changes: inflammation, going on to necrosis of the epithelium, multiple hæmorrhages, etc.

Atoxyl and Donovan's Solution.

Nine further experiments have been made with this combination, also in larger doses; but these larger doses have been invariably fatal, with lesions both of the intestines and kidneys. The doses were arranged upon the basis of the doses recorded, with such good results, by Drs. Moore, Nierenstein, and Todd;* but one of us has received a letter, since the experiments were completed, from Dr. Nierenstein, stating that the Donovan's solution used in their experiments was diluted with an equal part of water.

Atoxyl and Liq. Hydrarg. Perchlor.

A series of 12 rats was treated with this combination on the lines laid down in the paper above referred to, by Drs. Moore, Nierenstein, and Todd. The results obtained by them gave much hope that this combination would be especially useful. But we have not been able to get such good results. Out of 12 rats so treated only one is alive at the 97th day, the others having died with acute renal lesions. Of course, really comparative results are always difficult to obtain, and in rats the individual equation, with regard to dosage, and to resistance both to drugs and to disease, is a very varying one. We have, for instance, found, with the rats we have used, that the white ones are more susceptible both to diseases other than trypanosomiasis and to drugs than the black and white ones are; and we find the grey are the least susceptible.

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* "On the Treatment of Trypanosomiasis by Atoxyl . . . . . followed by a Mercurial Salt, etc.," 'Biochemical Journal,' vol. 2, Nos. 5 and 6.

From the above, considering both those experiments recorded in our former paper which have since ended fatally, and the more recent and—as regards dosage—bolder experiments, we are forced to the conclusion that, in small animals at any rate, mercury has not in our hands given altogether satisfactory results. Perhaps it may be a question of dosage; we have, however, tried to enlarge the range of dosage, as far as possible, from homœopathic doses to large ones, without attaining a large percentage of cures. If the dose of mercury be sufficient to aid the atoxyl, as in the cases brought forward from our last paper, we have found, in those cases which have died, chronic kidney, and in a less degree liver, lesions, which seem to be the late result of those more acute changes which we have found in those animals which have died earlier, either from disproportionate dosage or from some want of resistance to the drug.

Perhaps, in dealing with a more chronic trypanosome disease such as Sleeping Sickness in man, the results would be more favourable. We have, for instance, two Sleeping Sickness rats, inoculated on April 15, which have been treated with quite small doses of atoxyl and succinimide of mercury, and in which no trypanosomes have been found since May 4; they appear to be quite well. We have also another Sleeping Sickness rat, inoculated on May 6, which has been treated only with atoxyl, and in which no trypanosomes have been found since May 28. But in the more acute forms of trypanosomiasis, such as Nagana and Surra, the method of treatment by atoxyl and some form of mercury has, in our experience, almost invariably led to degenerative lesions, principally in the kidneys, which has been a cause of death long after any trace of trypanosomes could be found, when we have believed that the animal has been quite cured of the initial disease.

Tiodine.

A few rats were treated with this substance, which is thiosinaminethyl-iodide ($C_6SN_2H_{13}I$). In doses of 10 minims it is immediately fatal to rats, although it is stated to be non-poisonous to man, and in doses of 5 minims caused death within 24 hours; in smaller doses, alone or in combination with atoxyl, it had no influence on the disease.

Certain Antimony Compounds.

The treatment with arsenic compounds was, as has been stated, attended with only partial success. Professor Cushny, F.R.S., who has advised us on pharmacological matters throughout these investigations, sent us for trial a weak combination of glycine and antimony, attempts to form an antimony compound analogous to atoxyl having failed. When injected into inoculated rats, the antimony glycine was found to possess, in a less degree, the power of the antimony compounds described below; it reduced the number of trypanosomes, and caused their disappearance from the blood for a time, if they were not very numerous.

But the combination was difficult to make, varied in strength, and its solution was very dilute, and its use was abandoned when it was found that the other antimony compounds, described below, were so much more effectual.

Potassium Antimonyl Tartrate.

Potassium antimonyl tartrate (tartar emetic), which is easily soluble, was then tried. In doses of 1 c.c. of a 1 per cent. solution it proved fatal within 24 hours to four inoculated rats of weights varying from 190 to 225 grammes, but it was noticed that the trypanosomes had greatly diminished in number. It was also noticed that the rats appeared to be ill and faint for a short time after the injection: they were unsteady and sometimes rolled about. This was at the time attributed to the depressing effect of the potassium in the compound, and suggested the making and using of the substance described below, with which all of our experiments have so far been done. The question of the effect of this particular salt on the rats themselves will be mentioned later. Experiments are in progress for the purpose of comparing the actions of the potassium and sodium compounds: the latter would seem to possess some theoretical advantage, especially in treating larger animals, when the dose will have to be proportionally larger.

Sodium Antimonyl Tartrate.

Through the kindness of Dr. R. H. Aders Plimmer, of University College, we have been able to obtain and use this compound, which is the sodium salt corresponding to potassium antimonyl tartrate. He has prepared a quantity of the pure substance for us, in crystalline form, and has written the appended Note* upon its chemistry.

This substance in 1 per cent. solution is that which, of all the various bodies mentioned in these papers, including atoxyl, has the most marked and remarkable influence upon trypanosomes in the living body. Although our experiments with it are not many, nor of long duration, the results so far seemed sufficient to induce us to call the attention of other workers in this field to it.

The injection of this compound causes no pain, nor does it produce any inflammation of the tissues; and the results of the injection are very striking. The trypanosomes disappear with great rapidity from the blood, and in the majority of the cases treated so far there has been no recurrence; and inoculations made from animals which have been killed, or have died, have been invariably negative. It acts much more quickly than atoxyl, and its dose is very much smaller, and it does not produce any undesirable effects on the animals, and recurrences are very much less frequent than is the case when atoxyl has been used.

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* See Note at end of paper, p. 11.

We have used a 1 per cent. solution of the solid salt, but this is quickly invaded by moulds, and needs the addition of a crystal of thymol, or of 0·25 per cent. of formalin. The question of dosage is still under observation. We have tried many ways, and at present we are inclined to think that a full dose (*e.g.*, 0·5 c.c. of a 1 per cent. solution for a rat of 200 grammes or over) should be given when the trypanosomes are fairly plentiful in the blood, and then repeated at intervals of one, two, and three days, up to about four doses, and thereafter in weekly doses for a month. But we have good results in cases in which a dose has been given on four successive days, also when given every other day, and so on up to once every five days, without any recurrence up to as many as 52 days; but of two cases dosed at five-day intervals, one has recurred and one has not. As regards the quantity which can be taken, we have one rat of 130 grammes weight which has taken 0·5 c.c. of a 1 per cent. solution on the 3rd day (when trypanosomes were plentiful in the blood), and again on the 5th, 0·29 c.c. on the 6th and 7th days, and 0·25 c.c. on the 8th, 9th, 10th, 12th, 13th, 14th, 15th, 16th, 17th, and 19th days, and it is still living and well on the 43rd day, and has had no recurrence. When it is given in a full dose for the first time to rats whose blood is swarming with trypanosomes, the rat generally becomes very restless, and rolls about; its respirations become very quick, and it appears to be ill. These symptoms were noticed in the initial experiments with tartar emetic, and were attributed to the potassium contained in it, but we think that they are more probably due to the changes in the blood caused by the destruction or solution of the trypanosomes, which occurs very rapidly, as they do not occur after the second dose, when there are no trypanosomes. Recovery has, so far, always taken place from this condition, but it would seem advisable, in cases where the first dose is delayed until the blood is swarming with trypanosomes, to give the dose in two halves at intervals of a few hours.

The quickness of the action of sodium antimonyl tartrate is very remarkable. In one rat, whose blood was swarming with trypanosomes, a dose of 0·35 c.c. of a 1 per cent. solution caused their entire disappearance from the blood within half an hour; and in two other cases, in which the blood contained very large numbers of trypanosomes, after injection of 0·33 c.c. only a few could be found at the end of half an hour, and in one after an hour none could be found, and in the other only one in an ordinary blood preparation (see Plate 1). In these cases a few trypanosomes can sometimes be found in the liver, and these are extremely active and in no way inconvenienced by the drug; whether these are the forms which can persist, and need to be tired out by successive doses, we cannot say at present, but their extreme activity, when all the others have disappeared, is suggestive. We have up to the present not detected any morphological differences in them.

A striking instance of the power of this compound over trypanosomes is seen in the case of a guinea-pig which was inoculated with *Trypanosoma Gambiense* on April 9. From July 3 to September 16, trypanosomes were present in the blood, latterly in quantity, and the animal was dying on September 16; its eyelids were œdematous and nearly closed; it had œdema of the genitals and anus, and a discharge of bloody mucus from the rectum, and its hair was coming out in large patches. At this date—September 16—it was given 0·5 c.c. of a 1 per cent. solution; on the 17th the trypanosomes had entirely disappeared, and 0·75 c.c. was given; on the 19th the animal to all appearances was quite well, and on this day, and on 21st and 26th, 1 c.c. was given. The œdema disappeared and it continued to look well, and showed no more trypanosomes. It lived until October 14, when it died; *post mortem* the organs were congested and the kidneys were inflamed and the urine in the bladder contained albumen. The fact that the guinea-pig was moribund when the treatment was commenced may reasonably account for the pathological condition.

The following table shows the general results, as obtained so far, of the treatment with sodium antimonyl tartrate. Of the 39 rats enumerated in this table, the first 3 had been treated at first with antimony glycine; and of the 11 of the remaining 36 which have died, 6 did not die of the disease, and there remain alive and well 3 of 52 days, 1 of 49, 7 of 44, 8 of 43, 4 of 31, and 2 of 21; and of these 25, 23 have had no recurrence. It will be seen that 8 of the rats (4 Nagana and 4 Surra) have been treated with mercury in addition to the sodium antimonyl tartrate, in order to see if these obtained thereby any advantage over those not so treated. So far as we can tell at present, there seems to be no obvious advantage: one of the Nagana rats treated with liq. hydrarg. perchlor. has had three recurrences, and one Surra rat treated with succinimide of mercury has died after one recurrence.

Sodium Arsenyl Tartrate.

As the results with the sodium antimonyl tartrate were so definite, it seemed worth while to investigate the effect of the corresponding arsenic compound.

Table of Nagana and Surra Rats treated with Sodium Antimonyl Tartrate. Average duration of untreated diseases 5·5 and 6·9 days respectively.

Disease.	No.	Weight in grammes.	Total amount of 1 per cent. sodium antimonyl tartrate in c.c.	Result to Oct. 24.	Recurrences.	Remarks.
Nagana ...	1	150	6 ·2	Died on 49th day...	2	Antimony glycine had been given at first, sodium antimonyl tartrate on first recurrence.
"	2	200	0 ·83	" 25th " ...	1	" " " No trypanosomes were found for 12 days before or at death.
"	3	170	1 ·75	" 35th " ...	1	Antimony glycine had been given at first, sodium antimonyl tartrate on first recurrence. No trypanosomes found for 10 days before, or at death, and a mouse inoculated with bone marrow did not take the disease.
"	4	115	2 ·76	" 38th " ...	1	No trypanosomes found for 16 days before, or at death. Rat became paralysed.
"	5	175	1 ·35	" 16th " ...	1	Probably too small a dose.
"	6	125	1 ·0	" 18th " ...	2	" "
"	7	200	3 ·0	*Living 52 days*......	0	
"	8	150	2 ·5	" *52* "	0	
"	9	250	2 ·6	" *52* "	0	
"	10	215	3 ·5	" *49* "	0	
"	11	125	2 ·6	Died on 22nd day...	1	Died of septicæmia from sloughing tail, not of disease. No trypanosomes found.
"	12	195	3 ·25	" 19th " ...	0	" " "
"	13	175	1 ·85	" 40th " ...	0	Died of septicæmia from an abdominal abscess, not of disease. No trypanosomes found.
"	14	110	1 ·9	*Living 43 days*......	0	
"	15	130	3 ·6	" *43* "	0	This rat had 14 doses in 19 days, *vide* p. 6.
"	16	155	1 ·5	" *43* "	0	
"	17	120	1 ·15	" *43* "	0	
"	18	225	3 ·75	Died on 31st day ...	2	No trypanosomes found *post mortem*.
"	19	140	1 ·48	*Living 43 days*......	0	

„	20	125	3·75	„ *43* „	3	Had 2 × 0·5 c.c. liq. hydrarg. perchlor. after first four doses of sodium antimonyl tartrate.
„	21	150	1·5	„ *43* „	0	„ „ „
„	22	125	1·5	Died on 27th day...	1	Had 2 × 0·25 mg. succinimide of mercury after first four doses of sodium antimonyl tartrate.
„	23	125	1·1	*Living 43 days......*	0	„ „ „
„	24	150	2·5	Died on 28th day...	1	Did not die of disease. No trypanosomes found.
„	25	115	1·8	*Living 31 days......*	0	
„	26	125	2·1	„ *31* „	0	
„	27	125	2·1	„ *31* „	0	
„	28	140	2·0	„ *31* „	0	
„	29	190	1·5	„ *21* „	0	
„	30	150	1·4	„ *21* „	0	
Surra	31	275	5·0	*Living 44 days......*	1	
„	32	175	1·9	„ *44* „	0	
„	33	175	1·5	„ *44* „	0	Had 2 × 0·5 c.c. liq. hydrarg. perchlor. after four doses of sodium antimonyl tartrate.
„	34	175	1·5	„ *44* „	0	„ „ „
„	35	200	2·0	„ *44* „	0	Had 2 × 0·25 mg. succinimide of mercury after four doses of sodium antimonyl tartrate.
„	36	150	1·5	„ *44* „	0	„ „ „
„	37	175	3·4	Died on 37th day...	0	Rat had eaten part of its fore-leg and tail, and was killed. No trypanosomes had been seen since the fourth day of the disease (and first of treatment). None were found *post mortem*, and two rats were inoculated with gland and marrow, with negative result.
„	38	250	4·0	*Living 44 days......*	0	
„	39	250	2·0	Died on 11th day...	0	Did not die of disease. No trypanosomes found after fourth day of disease (and first of treatment).

Sodium Arsenyl Tartrate or Sodium Tartrarsenite.—This compound was investigated by Henderson and Ewing* in 1895, who gave it the formula $AsONaC_4H_4O_6.2\frac{1}{2}H_2O$.

It was prepared for these experiments by dissolving one equivalent of arsenious oxide in two equivalents of acid sodium tartrate, filtering, evaporating to a small volume, and adding alcohol till crystallisation commenced; on cooling, the substance crystallised out.

This substance does not seem to be anything like so effective as the antimony compound. Of five rats which have been treated with it, four died between the 12th and 24th days, and three of these had a recurrence; one is still living on the 21st day, but we think that this is probably due to the sodium antimonyl tartrate which was given after a recurrence, in the same way as if from the beginning of the disease.

A mixture of equal parts of a 1 per cent. solution of sodium antimonyl tartrate and of sodium arsenyl tartrate has been tried on six rats. One died on the 14th day and the five others are still living on the 21st day without any recurrence.

Immunity.

With a view of ascertaining what amount of immunity, if any, had been conferred on an animal which we considered to be cured, a Nagana rat was taken which was inoculated on May 13, and had been afterwards successfully treated with atoxyl and succinimide of mercury, and in which no trypanosomes had been found since it had its first dose on May 16, when the trypanosomes were very plentiful in the blood. On October 7, the 147th day, the rat was re-inoculated from another Nagana rat, and on the 11th trypanosomes were present in numbers in the blood; a dose of sodium antimonyl tartrate was given and no trypanosomes have been seen since the 12th. This seems to point to the fact that no immunity is conferred.

DESCRIPTION OF PLATE.

The microphotographs were made from rough blood-preparations, with a low-power objective (Zeiss 8 mm.), in order to demonstrate the rapid disappearance of the trypanosomes from the blood after administration of sodium antimonyl tartrate.

Fig. 1 shows the blood of a Nagana rat, 4 days after inoculation, *before treatment.*

Fig. 2 shows the blood from the same rat *half an hour* after the injection of 0·35 c.c. of a 1 per cent. solution of sodium antimonyl tartrate.

Fig. 3 shows the blood from the same rat *one hour* after administration of the above dose

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* Henderson and Ewing, 'Chem. Soc. Trans.,' 1895, vol. 67, p. 103.

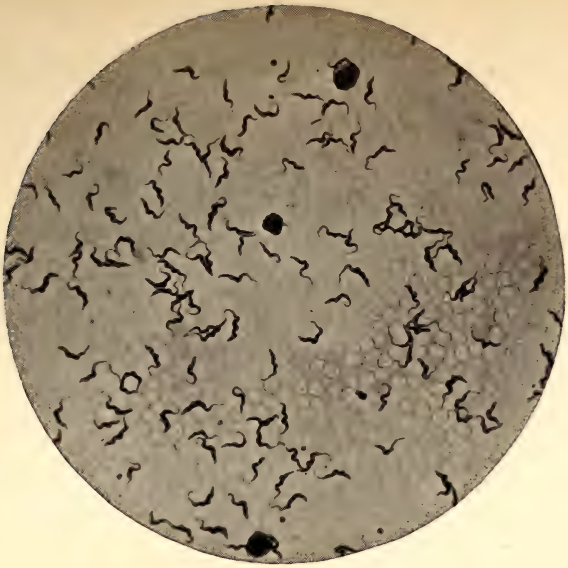

FIG. 1.

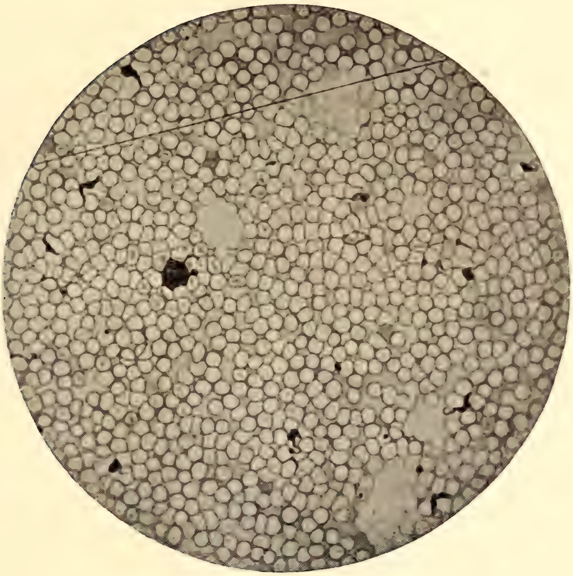

FIG. 2.

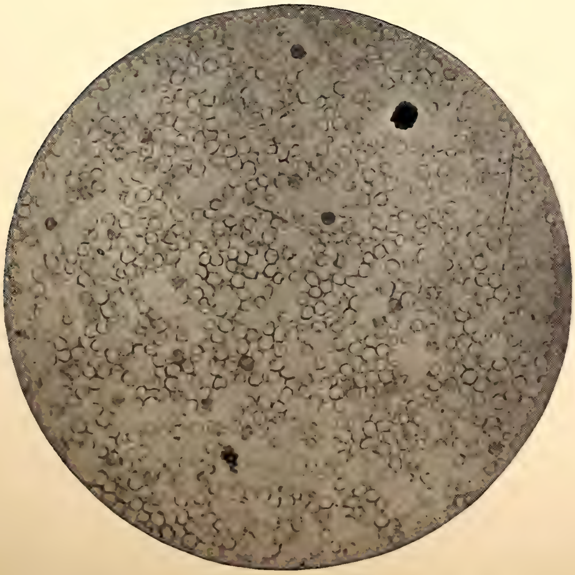

FIG. 3.

Note upon Sodium Antimonyl Tartrate.

By R. H. Aders Plimmer, D.Sc. Lond.

Sodium antimonyl tartrate was described in 1842 by Dumas and Piria,[*] who gave it the constitution $C_8H_8O_{10}NaO,Sb_2O_3H_2O$,[†] but did not state how they had prepared it. Clarke and Evans[‡] obtained a compound of the composition $3Na_2C_4H_4O_6 + 2Sb(OH)_3 + 3H_2O$, in 1883, by saturating tartaric acid with antimony trioxide and neutralising the solution with sodium carbonate. The first compound does not seem to have been prepared again since 1842.

Sodium antimonyl tartrate was prepared according to the methods usually given for preparing tartar emetic, by boiling a solution of acid sodium tartrate (13 grammes) with a little more than the calculated quantity (10 grammes) of antimony trioxide until the latter had almost completely passed into solution. On filtering and concentrating the solution to a small volume no crystallisation occurred, but on adding a little alcohol the whole became solid. This was then dissolved in about twice its volume of hot water, and alcohol was added until precipitation commenced, when, on cooling, the sodium antimonyl tartrate crystallised out. This compound at the ordinary temperature dries very slowly and has a moist appearance, but when dried *in vacuo* over sulphuric acid it becomes anhydrous and loses 2½ molecules of water of crystallisation, resembling sodium tartrarsenite in this respect. The substance is very easily soluble in water and its solution reacts faintly acid to litmus.

0·7228 gramme air-dried substance lost *in vacuo* over sulphuric acid 0·0920 gramme H_2O = 12·73 per cent.

1·0538 „ „ „ „ 0·1338 gramme H_2O = 12·70 per cent.

Calculated for $C_4H_4O_7NaSb.2\frac{1}{2}H_2O$. H_2O = 12·8 per cent.

I. 0·4058 gramme substance dried *in vacuo* over sulphuric acid gave 0·2332 gramme Sb_2S_3 and 0·0974 gramme Na_2SO_4.

II. 0·3780 gramme substance dried *in vacuo* over sulphuric acid gave 0·2072 gramme Sb_2S_3 and 0·0912 gramme Na_2SO_4.

Calculated for $C_4H_4O_7NaSb$. Sb = 39·09 per cent.; Na = 7·49 per cent.
Found :—I. Sb = 39·28 „ Na = 7·77 „
II. Sb = 39·14 „ Na = 7·81 „

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[*] Dumas and Piria, 'Liebig's Annalen,' 1842, vol. 44, p. 89.

[†] Old notation.

[‡] Clarke and Evans, 'Berichte,' 1883, vol. 16, p. 2385.

When dried at 105° C., the substance loses only two molecules of water of crystallisation :—

0·6120 gramme air-dried substance lost at 105° C. 0·0640 gramme H_2O = 10·46 per cent.

Calculated for $C_4H_4O_7NaSb.2H_2O$. H_2O = 10·49 per cent.

The remaining half molecule is subsequently lost *in vacuo* over sulphuric acid.

0·6120 gramme substance dried at 105° C. then lost *in vacuo* over sulphuric acid 0·0110 gramme H_2O. Total loss = 12·25 per cent.

On exposure to air, the two and a half molecules of water of crystallisation are again taken up, but the salt does not deliquesce.

0·5370 gramme substance dried *in vacuo* over sulphuric acid, on exposure to air ncreased in weight to 0·6066 gramme.

The Influence of Increased Barometric Pressure on Man. No. 4.—The Relation of Age and Body Weight to Decompression Effects.

By Leonard Hill, F.R.S., and M. Greenwood, Jun., M.R.C.S.

(Received October 1,—Read December 5, 1907.)

Statistics of caissons and diving works tend to suggest that the percentage number of men affected injuriously by exposure to compressed air increases with age.

Pol and Watelle (1) record that men between 18 and 26 stood the work best, and that of the 25 men dismissed for illness from the works under their inspection, 19 were over 40 years old, 5 over 30, and 1 over 28 years.

Catsaras (2) investigated 62 instances of paralysis among sponge divers, and we find that, of these, 33 were over 30 years old, 17 over 25, 11 over 20, and 1 over 19. These men dived about 140 feet, spent about 10 minutes below, and were decompressed in about one minute.

Evidently this variation might depend on—(i) the actual age difference; (ii) on an increase in mean body weight with age; (iii) on a combination of (i) and (ii); (iv) it might be purely random. We cannot absolutely exclude (iv) in the instance of caisson works unless we know the total number of men at each age employed, figures which do not seem to be available.

Snell (3) gives the following table (Table I) of his observations, made at the Blackwall Tunnel works.

Unfortunately, column 2 gives not the total number employed, but only those who submitted themselves to medical inspection, which was not, at first, compulsory.

Table I.

Ages.	Number of men examined and passed.	Number of men taken ill whose ages are recorded.	Proportion of illnesses to every 100 men passed.
15—20	55	0	0
20—25	145	15	10·3
25—30	152	37	24·3
30—35	91	19	20·9
35—40	61	14	22·9
40—45	38	10	26·3
45—50	3	5	166·0
Totals...	545	100	

Supposing, however, that the men examined by Snell were a fair sample of the workers, they can be used as a measure of the age distribution in the whole class of employés. If there be no special liability to illness at any particular age, the number of men of a given age who suffer should be simply $n'/\mathrm{N} \times$ total number of cases, where $\mathrm{N} = 545$, n' the number of men within the specified age limits as recorded above. In this way we obtain the subjoined table:—

Table II.

Ages.	Actual number affected.	Theoretical number.
15—20	0	10·09
20—25	15	26·61
25—30	37	27·89
30—35	19	16·70
35—40	14	11·19
40—45	10	6·97
45—50	5	0·55

Applying the usual test for goodness of fit (4) to this distribution, χ^2 is found to be 56·44, so that the odds are more than a million to one against a worse agreement between theory and observation if the deviations are merely a result of random sampling.

In the same way, if we group the 143 cases with recorded ages given by v. Schrötter (2), from the Nüssdorf Works, and assume Snell's age distribution to hold for these works (a not very improbable assumption, since we know that many caissoniers travel from place to place through England and Europe), we have:—

Table III.

Ages.	Actual number affected.	Theoretical number.
15—20	1	14·43
20—25	35	38·04
25—30	43	39·88
30—35	32	23·87
35—40	19	16
40—45	8	9·97
45—50	5	0·79

and χ^2 is 39·13, or the odds are about three in a million. It will be seen, therefore, that there is some evidence in favour of an age bias, but the statistics are not sufficiently detailed to give much information.

Returning to (i), (ii), and (iii), it is clear that we must have considerable difficulty in isolating (i) and (ii) under experimental conditions. Thus, in dealing with such animals as rats, it is not easy to obtain a large number of the same age but markedly different body weights. If we attempt to overcome the difficulty by comparing animals of different species, a new factor is involved, the importance of which cannot at present be estimated.

It must, therefore, be admitted that in the results about to be detailed the observed differences cannot be ascribed wholly to body mass; other changes associated with growth and decay may be influential, and we must certainly not compare directly animals of different species. With these reservations, regarding which more will be said later, we shall demonstrate an appreciable difference in liability to caisson disease when animals of different weights are employed.

It is known that the velocity of the circulation and rate of respiratory exchange in small mammalia is greater than in large animals, the relatively larger surface exposure in the former necessitating a higher rate of metabolism. Since the saturation of the tissues with gas, together with its removal from them, are functions of the blood, it follows that the processes should require less time in small than large animals. If, then, we expose large and small animals to the influence of compressed air for so long a time that both will contain large quantities of dissolved gas, a decompression rate dangerous for the former should be safe for the latter.

Our experiments have been carried out on rats, mice, cats, rabbits, and guinea-pigs.

(a) *Rats.*

In all experiments the animals were exposed to a pressure of +105 lbs. for periods varying from a-half to two hours; in most cases the exposure was for one hour. Decompression was effected in four and a-half to seven seconds. In all, 85 rats have been used—54 small, 31 large; of the small rats, 14, or 25·9 per cent., died; of the large animals, 24, 77·4 per cent., died. Unfortunately, all the rats were not weighed, but we have records of the weight in 65 cases, and although this is not a great many from the statistician's point of view, it is sufficient to make an analysis interesting. The following attempt has been made to measure the relationship between decompression effects and body weight.

As we have no quantitative scale of decompression effects, the most satisfactory method to employ would seem to be that of fourfold division, obtaining the coefficient of correlation in the ordinary way (5). We accordingly made the following table :—

Table IV.

Weight in grammes.	Recoveries.	Deaths.	Totals.
Below 124	28	13	41
Above 124	6	18	24
Totals	34	31	65

From this r was calculated to be $0{\cdot}625 \pm 0{\cdot}095$.

At first sight this would suggest a very close relationship between low weight and immunity from illness, but it is more than doubtful whether the fourfold method is applicable.

For this process to be valid it is necessary that the distribution should be normal; thus, the modal severity of caisson illness should be neither among the very slight nor the very severe cases. As a matter of fact, human statistics show that "bends," slight decompression effects, are vastly more frequent than severe or moderately severe paralysis, so that the severity distribution is, perhaps, skew, and r, as found above, ceases to be a satisfactory measure of association. We have, however, two more tests of statistical relationship, the correlation ratio (6)

$$\left(\eta = \frac{\text{Standard Deviation of Means of Arrays}}{\text{Standard Deviation of the Character}}\right)$$

and the coefficient of mean square contingency (C_1) (7). We can calculate η readily; the mean weight of all the rats was 122·87 grammes, the standard deviation 67·08; the mean of survivors was 91·62 grammes, of victims 157·15, and $\eta = 0{\cdot}489$.

As a matter of fact this value differs from r by less than three times the latter's probable error, but this does not prove linearity of regression,* nor can we apply Blakeman's test for linearity (8), since the scale of caisson illness is not quantitative.

Finally, the mean square contingency was determined by means of the grouping shown in Table V, and proved to be 0·544 ± 0·078.†

While, therefore, the exact value of the relationship between body weight and decompression effects is hardly expressible in terms of normal correlation, it can scarcely be less close than that implied by a contingency coefficient of at least 0·3; in other words, the two variables are quite definitely correlated. We have at present no means of extending this analysis to any other group of animals.

Table V.

Weight in grammes.	Survived.	Died.	Totals.
40— 60	5·5	0·0	5·5
60— 80	12·0	4·0	16·0
80—100	8·5	2·0	10·5
100—120	2·0	6·5	8·5
120—140	1·0	1·0	2·0
140—160	2·0	4·5	6·5
160—180	1·0	6·0	7·0
180—200	1·0	1·0	2·0
200—220	1·0	0·0	1·0
220—240	0·0	3·0	3·0
300—320	0·0	1·0	1·0
340—360	0·0	2·0	2·0
Totals	34·0	31·0	65·0

Observations falling at a class limit (*e.g.*, 60) were reckoned as 0·5 in each class.

(b) *Rabbits.*

Our experiments have not been numerous, but the following are suggestive :—

Exp. 5.11.06.—Three rabbits, one full grown, another of medium size, a third smaller one, were exposed to a pressure of 105 to 110 lbs. for 51 minutes, and decompressed in four and a-half seconds.

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* In any case, but little weight can be attached to the value obtained for η, since there are only two arrays available. It seemed best, however, to record the value for comparison.

† Deduced by multiplying the probable error obtained from the ordinary formula by 4/3. The complete formula of Blakeman and Pearson (9) gave a lower value, perhaps owing to an undetected slip in the rather involved arithmetical computation. The exact significance of the coefficient may be realised by comparing its value with those obtained by Pearson (7) for the contingency between undoubtedly related variables :—

Stature in father and son ... ... $C_1 = 0·513$
Hair colour in brothers ... ... $C_1 = 0·614$
Occupation in fathers and sons ... ... $C_1 = 0·6275$

See pp. 21, 22 (7).

The biggest rabbit went into convulsions in three and a-half minutes, and the medium-sized one was affected shortly after. On unscrewing the chamber both were found dead, exhibiting the usual *post-mortem* appearances. The smallest rabbit seemed normal.*

(c) *Cats.*

Exp. 27.7.06.—A pregnant cat and half-grown kitten were exposed to + 100 lbs. for 30 minutes, and decompressed in six seconds. The cat died in 20 minutes.

Autopsy.—Veins and arteries full of bubbles and froth. Lungs emphysematous and congested in patches, owing to air embolism.

Fœtuses.—Air bubbles in amniotic fluid, which frothed on pouring into a beaker; air in fœtal lungs and liquid contents of stomachs, none visible in the blood. The kitten, which appeared normal, was killed for examination within a minute of opening the chamber. No bubbles were seen. The bladder was full of urine, which frothed like champagne when poured into a beaker.

25.1.07.—An old cat and a half-grown cat were exposed to + 110 lbs. for 85 minutes, and decompressed in seven seconds. The old cat was found to be dead on opening the chamber, the young cat survived 20 minutes.

26.7.07.—An old cat and two kittens (half grown) exposed to + 100 lbs. for 30 minutes and decompressed in 10 seconds. The cat was found dead on opening the chamber, the two kittens survived.

Collecting our results on rabbits and cats, we have :—

Table VI.

Animals.	Died.	Survived.	Percentage mortality, with probable errors.
Young rabbits	5	9	35·7 ± 8·64
Old rabbits	2	1	66·67 ± 18·36
Young cats	6	6	50·0 ± 9·735
Old cats	6	0	100·0 ± 8·0 (?)

The difference in the first group is hardly sensible (30·97 ± 20·3), in the second, perhaps, significant (50 ± 12·6).

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* Although this animal had no decompression symptoms, it died four days later from pneumonia, produced probably by the rupture of the lung tissue, which occurs in many cases on sudden decompression, followed by infection.

If young animals have an advantage here also, it is, to say the least, not very marked, and we may reasonably conclude that we have to deal with a group in which the absolute body mass of all members is so great that the decompression period (four to seven seconds) is too short even for the least bulky subjects.

In addition to this question of absolute body mass, there appear to be quite marked variations in different species. Thus, it by no means follows that animals of species A will stand decompression better than much heavier animals of species B. So far as our observations go, the guinea-pig is a peculiarly unfavourable subject, even when young and light animals are used; in 10 experiments we have had eight fatalities.

12.7.07.—Nine guinea-pigs were exposed to + 105 lbs. for one hour, and decompressed in four seconds. Eight (weights, 144, 72, 77, 164·5, 75, 114·5, 108·5, 81 grammes) were dead, and the ninth (108 grammes) paralysed, but recovered completely in a few days.

We shall next give details of comparative experiments, which suggest the same conclusion.

Rabbits and Cats or Kittens.

26.7.06.—Four rabbits (about one month old), three kittens (about three weeks old) were exposed to + 100 lbs. for 30 minutes, and decompressed in seven seconds. Two rabbits died and one kitten.

21.11.06.—Half-grown cat (3 lbs. 2 ozs.) and large rabbit (3 lbs. 8 ozs.) were exposed to + 105 lbs. for 35 minutes. Decompression time, four seconds. The cat was dead on removal, the rabbit unaffected.

Rabbits and Guinea-pig.

10.5.07.—Two young rabbits (3 lbs. 6 ozs.; 3 lbs. 2 ozs.), one guinea-pig (1 lb. 8 ozs.) exposed to + 100 lbs. for 30 minutes, and decompressed in seven seconds.

The rabbits died within 15 minutes, and the guinea-pig died in the night, showing signs of pulmonary hæmorrhage.

Rats and Rabbits.

10.7.07.—Two rats (435 grammes, 87 grammes), one rabbit (1304 grammes) exposed to + 105 lbs. for $1\frac{1}{2}$ hours, and decompressed in four seconds.

All were dead on removal.

Evidently these results are indecisive.

Rats and Mice.

7.12.06.—Six rats and six mice were exposed to + 115 lbs. for 25 minutes, and decompressed in four seconds. Four rats died (162, 104, 120, 152 grammes), two (107, 87 grammes) survived. All the mice survived (18, 18, 12, 10, 11, 10 grammes).

24.1.07.—Fifteen mice and five half-grown rats were exposed to + 115 lbs. for two hours, and decompressed in five seconds. Three rats and 15 mice were unaffected. One rat was dead (froth in heart and veins), one paraplegic.

In all our experiments on rats and mice, we find:—

	Died.	Survived.	Percentage mortality.
Rats	6	10	37·5 ± 8·16
Mice	3	27	10 ± 3·69
Difference	—	—	27·5 ± 8·96

The evidence in favour of the view that mice can stand rapid decompression better than rats is reasonably strong. Here body weight is apparently more important than age, full-grown mice being better subjects than young rats.

Rats and Kittens.

22.5.07.—Two full-grown rats (11 and 12½ ozs.), and three young kittens (11, 11, 10 ozs.) were exposed to + 120 lbs. for 55 minutes. Decompression time, 5½ seconds. One rat was dead on removal; two kittens and the other rat died in a few minutes; one kitten survived. P.M.: The air emboli usually observed were present in all three animals.

This experiment shows little advantage on the side of the young animals.

The results we have described may be compared with the observations of earlier workers which we have collected and tabulated. These seem to show a relative immunity of the smaller animals to rapid decompression, and a death rate of almost 100 per cent. in the case of cats and dogs at pressures above six to seven atmospheres.

Table VII.

Pressure in atmospheres.	Period of decompression.	Result.
Sparrows (Bert) (10).		
7	A few seconds	Death.
8 (for 5 mins.)	„	Nil.
8 (for 2 mins.)	„	Nil.
8 (for 2 hrs.)	„	Death.
9½ (1 hr. 35 mins.)	„	Nil.
10 (some minutes)	„	Nil.
12 „	„	Death.
14 „	„	No immediate result; died next day.
14 „	„	Death.
15 „	„	Death.
Mice (Phillipon) (11).		
5 (20 mins.)	1 min.	Nil.
12 (1 hr.)	1—2 secs.	Nil.
5 (1 hr.)	Momentary	Death.
Rats (Bert) (10).		
5½ (1 hr. 15 mins.)	A few seconds	Nil.
6½ (1 hr. 45 mins.)	„	Nil.
6½ (¾ hr.)	„	Nil.
8½	2 mins.	Death (2 animals).

Table VIII.

Pressure in atmospheres.	Period of decompression.	Result.
Rabbits (Bert) (10).		
6½ (1 hr. 45 mins.)	4½ mins.	Nil.
7 (a few minutes)	2—3 „	Nil (2 animals).
8 (5 mins.)	2—3 „	Nil.
8½	2—3 „	Nil (2 animals).
Rabbits (Phillipon) (11).		
4 (1 hr.)	Momentary	Death.
5½ „	„	Death.
5·6 „	„	Death.
7 (5 mins.)	„	Nil.
7 (1 hr.)	15 mins.	Slight symptoms; recovered.
Cats (Bert) (10).		
8 (5 mins.)	2—3 mins.	Paralysis and death.
10 (9 mins.)	2—3 „	Death.
10	2—3 „	Paralysis.

Table VIII—*continued.*

Pressure in atmospheres.	Period of decompression.	Result.
Dogs (Bert) (10).		
3½	1—2 mins.	Nil.
4 (15 mins.)	2—3 ,,	Nil.
4½	2—3 ,,	Nil.
5 (30 mins.)	2—3 ,,	Nil.
5½ (4 hrs.)	20 secs.	Nil.
6 (30 mins.)	20 ,,	Nil.
6 (2 hrs.)	20 ,,	Nil.
6 (a few minutes)	20 ,,	Slight paralysis.
6 (3½ hrs.)	20 ,,	Death.
6½ (a few minutes)	4½ mins.	Nil.
7 ,,	2 ,,	Paraplegia and death.
7 (7 mins.)	2 ,,	Paraplegia and death.
7 (10 mins.)	2½ ,,	Paralysis and death.
7½ (15 mins.)	2 ,,	Paralysis and death.
7 (a few minutes)	2¼ ,,	Paralysis and death.
7¼ ,,	1¼ ,,	Paralysis and death.
7¼ ,,	1¼ ,,	Paralysis and death.
7¼ ,,	2 ,,	Paralysis and death.
7½ ,,	2 ,,	Slight paralysis.
7½ ,,	2 ,,	Paralysis and death.
7½ ,,	2 ,,	Nil.
7¾ ,,	3 ,,	Paralysis.
8 ,,	3—4 ,,	Paralysis and death.
8¼ ,,	3 ,,	Paralysis and death.
8¼ ,,	3 ,,	Paralysis and death.
8½ ,,	2½ ,,	Paralysis and death.

Table IX.—Catsaras' Observations on Dogs (12).

Water depth.	Atmospheres (approximate).	Exposure.	Decompression time.	Result.
metres.			secs.	
34 ·0	4½	16 mins.	40	Nil.
34 ·0	4½	10 ,,	50	Nil.
38 ·0	4¾	21 ,,	60	Nil.
40 ·0	5	6 ,,	60	Nil.
43 ·0	5⅓	4 ,,	60	Nil.
34 ·0	4½	2½ hrs.	40	Temporary paralysis.
36 ·0	4½	2 ,,	50	,, ,,
38 ·0	4⅔	1¾ ,,	60	Death.
40 ·0	5	1 ,,	60	Paralysis and death.
43 ·0	5⅓	1 ,,	60	Dyspnœa ; recovered.
43 ·7	5½	2 ,,	60	Nil.
43 ·7	5½	1½ ,,	60	Paralysis.
47 ·2	5⅔	1 ,,	50	Temporary paralysis.
53 ·2	6⅓	½ ,,	40	Paraplegia.
55 ·4	6½	25 mins.	60	Death.
60 ·0	7	30 ,,	60	Death.
45 ·0	5½	30 ,,	30	Paraplegia.

Further, the observations of L. Hill and J. J. R. Macleod led them to conclude that all adult dogs and cats died when decompressed in a few seconds after an hour's exposure (or more) to eight atmospheres.

If the relative immunity of small animals be due to the greater velocity of the circulation in them, it follows that any agent which damages or slows the circulation would deprive them of their safeguard. We have tested this by exposing small animals to air pressure and then pumping chloroform into the chamber, or upsetting a bottle containing the anæsthetic placed inside.

8.5.07.—Guinea-pig (1 lb. 4 ozs.) placed in chamber with $CHCl_3$. At 11.30 A.M., pressure raised to + 100 lbs.; 11.35, beginning to show signs of anæsthesia; 12.12, decompressed from + 75 lbs. in five seconds. Dead on removal. P.M.: Heart auricles contained an enormous quantity of froth. Every vessel examined full of bubbles. Lungs almost bloodless in appearance. Renal vessels frothed on incision. Gall bladder, greatly distended, bile frothed almost like soda water.

Control 10.5.07.—Guinea-pig (1 lb. 8 ozs.) exposed to + 100 lbs. for 30 minutes, and decompressed in seven seconds. The animal seemed normal on decompression, but was found dead next day (Saturday). P.M. (Monday): There were signs of pulmonary hæmorrhage, but the body was decomposing.

15.5.07.—Three young rats (4 ozs., 3 ozs., 2 ozs.) and a kitten were compressed at 12.50 P.M. to + 110 lbs. At 2.8, chloroform was pumped into the chamber, and at 2.11, when they were all lightly anæsthetised, decompression was effected in five and a-half seconds. On opening the chamber the kitten and one rat were found dead. The other two rats died in a few minutes. P.M.: All exhibited an enormous amount of gaseous embolism except the rat which survived longest, and even in this case many emboli were seen, especially in the vena cava inferior.

These experiments, especially the last, are in favour of the view that immunity does depend on a rapid circulation.

In considering the practical bearing of our results, it is to be remembered that the conditions which enable a small animal rapidly to discharge an excess of dissolved gas also lead to its more rapid saturation. Hence, *short* exposures are relatively more dangerous for small than large animals. The advantages of small body mass and youth should accordingly be more apparent among caisson workers than in diving operations; we are not acquainted with any statistics bearing on this point.

It is interesting to note that we have no reason to think that small animals are more susceptible to oxygen poisoning than large ones. The following observations of L. Hill, J. J. R. Macleod, and C. Ham are suggestive of this:—

Table X (13).

Animal.	Atmospheres of air.	Period of exposure.	Result.
Mouse	8	27 hrs.	Pneumonia.
Mouse	11	1½ ,,	Death.
Cat and 4 kittens	8—6	24 ,,	Pneumonia.
Dog	8	24 ,,	Gasping respirations; recovered.
	Atmospheres of O_2 (about 90 per cent.).		
Mouse	6	2½ hrs.	Pneumonia.
Rabbit	6	1½ ,,	,,
Rat	6	1½ ,,	,,
Cat	6	5½ ,,	,,
Mouse	5	12 mins.	Convulsions.
Mouse	5—6	32 ,,	,,
Mouse	6½	5 ,,	,,
Rat	6	32 ,,	,,
Rabbit	6	17 ,,	,,
Cat	6	3 hrs. 30 mins.	,,
Cat	4—5	15 mins.	Nil.
	7	3½ ,,	Convulsions.
Cat	5	3½ ,,	,,
Cat	4½	6 ,,	,,
Cat	2⅔	1 hr. 20 mins.	,,
Young rabbit Young mouse Small mouse (16 grammes) Large mouse (26 grammes) Small rat (50 grammes)	2⅔ 4⅓	1 hr. 40 mins. 15—20 mins.	Nil. All the animals convulsed.

In the case of the lungs the action of the oxygen is direct, and there is no reason to suppose that the size of the animals should have any influence here. As to the convulsions, Lorrain Smith (14) has shown that these are caused not by the amount of oxygen combined, but by the tension of oxygen dissolved in the blood. Convulsions are as readily produced in an animal whose blood is half saturated with carbon monoxide as in the normal state. The immediate factor in their causation is unknown.

Conclusions.

1. Small mammals are relatively immune from decompression effects.

2. This immunity depends on rapidity of circulation, and may be destroyed by damaging the latter with chloroform.

3. Age is probably important *per se,* but of far less importance than body weight. We have no convincing proof that two animals of the same weight but different ages would exhibit unequal resisting powers.

4. There is no evidence that small animals are more quickly poisoned by high pressures of oxygen than large ones.

The practical outcome of this research is that young men of small body weight and possessing a vigorous circulation should be selected for compressed air works.

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On the Distribution of the Different Arteries supplying the Human Brain.

By Charles E. Beevor, M.D. Lond., F.R.C.P.

(Communicated by Professor David Ferrier, F.R.S. Received May 8,—
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(Abstract.)

The present work was undertaken to ascertain the area of distribution of the different arteries of the brain, when they were injected simultaneously under the same pressure with gelatine containing soluble colours.

The number of brains injected is 87. The arteries injected were the posterior communicating, the anterior choroid, the anterior cerebral, the middle cerebral, and the posterior cerebral.

The *previous observations* of Duret, Heubner and Kolisko and other authors are described.

The *method of investigation* consisted in injecting simultaneously by means of pressure bottles three, four, or five of these arteries with different soluble colours. The injection mass used was gelatine, coloured with soluble carmine, Nicholson's blue, naphthol green, acridine yellow, and Bismarck brown. Twelve different classes of experiments are described. The brains were hardened in formalin, and subsequently cut and examined in the sagittal, horizontal, or coronal planes.

The most frequent distribution of the basal branches to the different parts is by the following arteries:—

Regio subthalamica, including the corpus subthalamicum and Forel's field, by the posterior communicating.

Corpus mammillare, by the posterior cerebral.

Pes pedunculi, anterior one-third by the posterior communicating or anterior choroid, posterior two-thirds by the posterior cerebral.

Internal capsule, anterior division, by the anterior cerebral in the inferior half and by the middle cerebral in its superior half. Posterior division, the inferior part, the anterior one-third by the posterior communicating, posterior two-thirds by the anterior choroid, which also supplied the retro-lenticular fibres; the superior part by the middle cerebral.

Caudate nucleus, caput, inferior half by the anterior cerebral, superior half by the middle cerebral. *Superior horizontal part* by the middle cerebral, posteriorly by the posterior cerebral. *Surcingle* by the anterior horoid.

Lenticular nucleus, external segment, anterior inferior part by the anterior cerebral, the rest by the middle cerebral. *Middle segment* by the middle cerebral. *Internal segment* by the anterior choroid.

Optic thalamus, anterior nucleus, by the posterior cerebral. *External nucleus* anteriorly by the posterior communicating, posteriorly by the posterior cerebral. *Internal nucleus* by the posterior cerebral, anterior half sometimes by the posterior communicating. *Pulvinar* by the posterior cerebral.

Choroid plexus in the descending and posterior cornua by the anterior choroid, in the central part of the lateral ventricle by the posterior cerebral.

Choroid membrane in the descending cornu, anterior part by the anterior choroid, the rest of the membrane by the posterior cerebral.

Optic tract by the anterior choroid.

Anterior commissure in the median part by the anterior cerebral, in the outer part by the middle cerebral, and in the postero-external part by the anterior choroid.

On the cortex the areas of supply of the chief arteries differ in their extent from the description given by Duret, and are most commonly as follows:—

Anterior cerebral artery, on median surface of cortex, to half way along the quadrate lobule; on external surface, posteriorly to half way along the parietal lobule, inferiorly to the sulcus frontalis superior.

Middle cerebral artery, on outer surface, posteriorly to the occipital pole or half an inch anterior to it, superiorly to the middle line along the posterior half of the parietal lobule, inferiorly to the middle of the inferior temporal gyrus; on median surface to include the hippocampal lobule.

Posterior cerebral artery, on median surface, anteriorly and superiorly to the posterior half of quadrate lobule; on external surface at the median line anteriorly to the external parieto-occipital fissure, posteriorly to the occipital pole or to half an inch anterior to this, and inferiorly to the middle or superior border of the inferior temporal gyrus.

The *optic radiations* in the occipital lobe are supplied in the upper (vertical) three-fourths by the middle cerebral and in the inferior one-fourth by the posterior cerebral artery, except in the posterior inch, where all by the posterior cerebral.

The following parts are also supplied by the cortical arteries:—

The *corpus callosum,* the genu, rostrum and body by the anterior cerebral; the tapetum by the anterior, middle or posterior cerebral; the splenium and forceps major by the posterior cerebral artery.

The *uncus* by the anterior choroid artery.

The *cornu ammonis* by the anterior choroid artery or the posterior cerebral artery.

The *centrum ovale* by the anterior, middle, and posterior cerebral arteries.

The simultaneous injection of the five arteries supplying the brain, by the same pressure, with soluble colours in gelatine has (it is believed) not been accomplished before, and it is considered that this method gives the most accurate representation of the distribution of each artery, including that of the finest capillaries.

The parts of the brain, the arterial supply of which hitherto has not been described, or which are found to be different from other observers, are:—

The regio subthalamica, with the corpus subthalamicum and Forel's field, the supply to which has not been described; the relative supply of the arteries to the pes pedunculi; the corpus mammillare (by the posterior cerebral in place of the posterior communicating); the exact supply to the anterior limb of the internal capsule; the absence of anastomoses of the three arteries supplying the posterior limb of the internal capsule; the exact supply to the caudate nucleus; the absence of anastomosis in the head of the caudate nucleus between the anterior and posterior cerebral arteries; the exact supply of the lenticular nucleus; the supply to the different nuclei of the optic thalamus, which has not been described before (the thalamus is not supplied by the lenticulo-optic arteries of Duret); the anterior part of the choroid membrane, which is supplied by the anterior choroid artery; the supply to the fornix and the anterior commissure.

In the cortex the observations differ from those of Duret in that, though there are great varieties, the anterior cerebral area extends most frequently on the outer surface along the median line posteriorly to midway between the Rolandic fissure and the external parieto-occipital fissure, instead of to the Rolandic fissure; and inferiorly to the sulcus frontalis superior, instead of the sulcus frontalis inferior. The middle cerebral area on the outer surface reaches the middle line for the posterior half of the parietal lobe, in place of along the whole extent of the lobe; and posteriorly the posterior pole, or half an inch in front of it, in place of the upturned end of the parallel sulcus; and inferiorly the middle of the third temporal gyrus in place of the middle of the second temporal gyrus; this diminution of the extent of the posterior cerebral area on to the outer surface corresponds to the increase of the middle cerebral area.

In the supply to the pedunculus cunei and the optic radiations the results agree with Henschen rather than with Monakow, though they differ from both in the case of the supply to the optic radiations; the supply to the fasciculus longitudinalis inferior is different to that given by Monakow, and corresponds to that of the optic radiations.

The exact arterial supply to the corpus callosum, uncus and cornu ammonis is given. And also that to the centrum ovale as seen in the sagittal, horizontal, and coronal planes.

The knowledge of the exact part of the brain which is supplied by any artery is of great importance in the diagnosis of the parts of the brain which undergo softening when this particular artery is blocked by a blood clot.

On the Cranial and Facial Characters of the Neandertal Race.

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(Received May 15,—Read November 14, 1907.)

(Abstract.)

As a result of a detailed comparison of the calvarium of the Neandertal race with that of the aborigines of South Australia, it is shown that a much closer resemblance exists between them than has hitherto been supposed, especially as regards the calottal height, calottal index, Schwalbe's ("bregma") angle, the bregma index, the frontal and orbito-frontal angles, the superior ("lambda") and inferior inion angles, and the fronto-parietal index. The chief differences are to be found in the magnitude of the cephalic index, the continuity of the frontal torus, and the deeply impressed character of the frontal fossa.

Comparisons based on the glabella-inion line are misleading, owing to the inconstancy in position of the inion. In the absence of any fixed point in the skull, the centre of figure of the median longitudinal section is chosen for a point of reference, and a radius drawn from this to the basion as an initial line for the measurement of polar co-ordinates.

The exterior foramino-basal angle owes its perplexing anomalies to the fact that its magnitude is determined by five independent variables, one of which is connected with the cranial height, so that in depressed forms of skull it acquires a higher value than might otherwise be expected.

The Gibraltar skull, preserved in the Royal College of Surgeons, is the only example of the Neandertal race which presents the bones of the face and the basi-cranial axis in undisturbed connection with the calvarium. Its characters, apart from the cranial vault, are unique: no other known skull possesses so long a face, such a large and broad nasal aperture, or such projecting nasal walls. In profile, the nasal curve flows into that of the glabella,

without any sudden change of flexure, that is, there is no nasal notch, such as occurs in the Australians.

The orbit, as in all skulls of the Neandertal race, is distinguished by its excessive height above a line drawn from the nasion to the middle of the fronto-zygomatic suture: in a low South Australian skull this height amounts to between 8 and 10 mm., in the Gibraltar skull to between 12 and 14 mm., and in the Neandertal calotte to between 19 and 20 mm. A further character of the orbits is the absence of a well-defined lower margin.

The absence of a canine fossa has been remarked upon by Huxley.

In the absence of the prosphenion and ephippium, the sphenethmoidal angle has been measured from the limbus sphenoidalis by a line drawn to the crista galli on the one hand and the basion on the other: it exceeds the corresponding angle of the lowest known South Australian skull, similarly measured, by 16° 30′.

The palate is parallel-sided and very dolicho-uranic. The thickness of the frontal bone, measured on one side of the crista galli, is 24 mm. The prognathism of the upper jaw, in whatever way it is measured, is extremely small, so that according to existing nomenclature the skull would be classed as orthognathous.

The cranial capacity is estimated as 1250 c.c., and thus makes a close approach to that of the Neandertal calotte. The average capacity of South Australian skulls is very similar, but ranges from 1460 to 1100 c.c. If the calotte of Pithecanthropus represents the mean of a similarly variable race, then the extreme forms of such a race would almost completely bridge over the hiatus between man and the higher apes.

On the Supposed Extracellular Photosynthesis of Carbon Dioxide by Chlorophyll.

By Alfred J. Ewart, D.Sc., Ph.D., F.L.S.

(Communicated by Professor J. Bretland Farmer, F.R.S. Received October 11,—Read December 5, 1907.)

In two recent papers, Usher and Priestley have brought forward evidence to show that chlorophyll is able to assimilate carbon dioxide and produce formaldehyde outside the plant, and that hydroxyl is the other product and is decomposed into water and free oxygen in the presence of a particular ferment. It is, unfortunately, necessary to point out that their work is to some extent vitiated by certain oversights and by one serious inaccuracy.

In their first paper* they confirm Bach's statement that formaldehyde is produced when light acts on a solution of uranium in the presence of carbon dioxide and water, although formic acid is much more abundant. In a later paper† they contradict this result, stating that formic acid alone is present and no formaldehyde, thus confirming Euler's‡ criticism of Bach's results.

Usher and Priestley consider that formaldehyde is an intermediate product, but bring forward no satisfactory proof. Their most striking experiment on extracellular photosynthesis is that made by painting a solution of chlorophyll on gelatine films and exposing them to light in the presence of carbon dioxide. The chlorophyll was bleached, which Usher and Priestley consider to be due to the formation of hydrogen peroxide, being apparently unaware of the fact that chlorophyll is bleached by sunlight in the presence of ordinary free oxygen or in air deprived of all carbon dioxide, and that the absence of hydrogen peroxide from living cells has been definitely established in a number of cases.§

In regard to the apparent detection of formaldehyde in the gelatine films, this is not surprising, because they contained in all probability an "aldehyde" substance before the chlorophyll was painted over them, and further show the same amount whether the films are exposed to light in the presence or absence of carbon dioxide. All the forms of French leaf gelatine (Coignet's) of cooking (transparent and opaque), Nelson's, and of impure commercial gelatine turn pink in the presence of a decolorised solution of rosaniline, with or without gentle warming, and it is on this reaction that Usher and Priestley mainly rely.

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* 'Roy. Soc. Proc.,' B, vol. 77, 1906, p. 369.

† *Loc. cit.*, B, vol. 78, p. 368.

‡ 'Ber. d. Deutsch. Chem. Ges.,' 1904, vol. 37, p. 3415.

§ *Cf.* Pfeffer's 'Physiology,' Eng. edit., vol. 1, pp. 545—546.

The best mode of obtaining the reaction is to soak the gelatine in water, melt, cool, and then pour on a little of the decolorised rosaniline, when a pink colour appears near the surface of the gelatine, even if an excess of sulphur dioxide is present. It must, however, be remembered that the same reaction is given by acetaldehyde, and possibly by other aldehydes also. Hence, possibly, may arise the fact observed by Usher and Priestley that the methyleneaniline obtained from the distillate from the gelatine had a slightly different melting point to that of the pure substance.* Even prolonged soaking and washing in water does not remove from the gelatine its power of assuming a pink or violet-pink colour with decolorised rosaniline, and it is quite evident that this oversight renders Usher and Priestley's method valueless. Repeating their experiments, however, there appeared to be a slight but distinct increase in the amount of "aldehyde" after the chlorophyll on the film had been bleached by exposure to sunlight, but quantitative estimation was not possible, and the same increase appeared to be shown by films exposed to light in the absence of carbon dioxide.

Usher and Priestley state that chlorophyll films on water also produce formaldehyde when exposed to light in the presence of carbon dioxide. I find this method extremely difficult to apply, and have not been able to obtain more than a suggestion of the possibility of the presence of formaldehyde in the subnatant water by the magenta test, and the presence or absence of carbon dioxide appeared to be immaterial. Hence a trace of formaldehyde might have been present in the original chlorophyll, the green colour of which masked the test when first applied.

Conclusive results were, however, obtained by using the paler bases of *Vallisneria* leaves. These showed no trace of formaldehyde when freshly examined, whether killed by heat, chloroform, or ether, but developed a strong pink coloration when placed in decolorised magenta after eight hours' exposure to sunlight. All green parts gave similar results, although wherever a tissue is dark green it is difficult to be sure of the absence of a trace of formaldehyde in the original material. Owing to the poisonous character of this substance, however, the amount originally present could not be large. After exposure to light, leaves of *Vallisneria* give the reaction rather better than leaves of *Elodea* and shoots of *Chara*. In the case of grass and other leaves, they must either be crushed or chopped after bleaching in sunlight, or the epidermis removed in order to allow the reagent to penetrate, otherwise the pink colour is only produced at the cut edges of the leaf.

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* Usher and Priestley, *ibid.*, p. 320.

The chloroplastids in the cells, after exposure and testing, show mostly a strong violet tinge when examined, but a slight tinge can usually be distinguished in the protoplasm, so that apparently the formaldehyde diffuses somewhat from the chloroplastids to the protoplasm, and, further, is not formed in all the chloroplastids even of one and the same cell. Furthermore, precisely the same production of "formaldehyde" is shown when the green tissues are exposed to light in an atmosphere free from carbon dioxide. It might be suggested that possibly a *post-mortem* production of carbon dioxide took place. Accordingly, leaves of *Vallisneria, Elodea,* and *grasses* killed by chloroform, ether, and heat were enclosed in vessels on clean strips of glass and kept in darkness for two and for 12 hours. The vessel contained a solution of caustic soda, and half of the inner wall was lined by a sheet of asbestos soaked in caustic soda. On then exposing to light until the leaves were nearly or completely bleached, all of them without exception developed a pink coloration with decolorised magenta, and the colour was as strong as that developed in the presence of carbon dioxide. In similar fresh leaves no formaldehyde whatever could be detected. It is evident, therefore, that Usher and Priestley have misinterpreted the facts, and that chlorophyll, when exposed to light in the presence or absence of carbon dioxide, yields an aldehyde, presumably formaldehyde, merely as a decomposition product. Interesting and suggestive as this fact may be, it affords no proof that formaldehyde is a product of photosynthesis, or that the latter can take place outside the living cell. The idea has more than once been put forward that chlorophyll itself is a product of photosynthesis, and that carbohydrates are formed by molecular changes and rearrangements in it taking place under the action and with the aid of light energy.

On examining parts of leaves growing in nature, but which had become bleached or discoloured, traces of formaldehyde appeared to be present in many cases, whether examined in the early morning or evening, and presumably in these cases also the aldehyde results from the decomposition of the chlorophyll.

Usher and Priestley state that on floating the white petals of *Saxifraga Wallacei* on water in the presence of carbon dioxide and sunlight, and after painting the petals with chlorophyll, the plastids in the cells developed starch grains, and they consider this to prove an extracellular decomposition of carbon dioxide outside the petals. In any case it would only prove that the traces of formaldehyde produced by the decomposition of the chlorophyll can be synthesised by the plastids to carbohydrate, and the experiment is open to two very serious objections. Firstly, the petals are cuticularised, and formaldehyde diffuses only very slowly through cuticle, even when comparatively thin. Secondly, except in the case of Compositæ the plastids of most white petals have faint but distinct traces of chlorophyll, either along the veins or in all parts. By *Saxifraga Wallacei*, Usher and Priestley presumably mean *S. Camposii* Boiss, living petals of which plant are not procurable in Melbourne, but they would find it worth while to examine the petals for traces of chlorophyll or for a feeble power of independent photosynthesis in parts at least. That this explanation is the correct one is shown by the fact that the petals formed starch in a 0·001-per-cent. solution of formaldehyde when exposed to light, but not in darkness, whereas if the carbohydrate had been derived directly from the formaldehyde, without any photosynthesis of carbon dioxide, it should have been formed both in light and darkness.

An equally strong objection applies to the experiment with *Elodea*, which formed starch and gave off oxygen in a 0·02 solution of formic acid in light, but not in darkness, the explanation being that the formic acid decomposed and the carbon dioxide produced was assimilated.

Hydroxyl and Peroxidase or "Catalase" Enzymes.

No conclusive proof has been brought forward to show that living plant cells even contain any appreciable amount of peroxide of hydrogen, and Pfeffer has conclusively shown in many cases, by the study of cells containing oxidisable pigments or chromogens, that no trace of hydroxyl is formed in them at any time. Russell* explains the action of plant tissues on photographic plates in the dark by assuming that they contain minute traces of hydroxyl, although it is not easy to see how this explanation could apply to a leaf which had been kept dried for 18 months to 3 years and still retained its power of affecting a photographic plate in darkness. There are many other possible explanations of the action, and hence by itself it affords no proof of the presence of hydroxyl. The oxidation of certain organic compounds yields hydrogen peroxide as a by-product, but in the living plant, as Loew† has shown, a catalase enzyme is generally present whose functions may possibly be to prevent the possibility of the accumulation of any hydroxyl, and the existence of such an enzyme was first noted by Schoenhein‡. The presence of a "catalase" enzyme is, however, no proof that a plant contains or produces hydrogen peroxide, for ferments have been isolated from many plants to which they cannot be of any value (rennet and diastase ferments in fungi), or have a very doubtful one (peptic enzymes in latex). Usher and Priestley do not mention Loew's work, and possibly were not aware of its existence, for Loew discusses the properties, forms, and distribution of his catalase very fully.

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* 'Roy. Soc. Proc.,' B, vol. 78, p. 385.

† Loew, 'U.S. Dept. Agric. Report,' 68, 1901.

‡ 'Journ. Prakt. Chem.,' vol. 89, 1863.

The Evolution of Oxygen.

By smearing gelatine chlorophyll films with a catalase enzyme, Usher and Priestley obtained an evolution of oxygen in the presence of carbon dioxide and light. I have not been able to obtain any satisfactory proof of this fact, and must point out that gelatine films smeared over with an oily film retain oxygen for some time and only give it off slowly in an atmosphere of nitrogen. After covering with chlorophyll and a catalase juice from *Vallisneria*, the films were kept in an atmosphere of nitrogen over mercury, a little carbon dioxide added and, after exposure to light, the gas was analysed in a Bonnier-Mangin apparatus. Barely measurable traces of oxygen were present in some cases, but hardly more than can be explained on the above assumption, and the amount of carbon dioxide underwent no appreciable diminution, but if anything a slight increase. Further, if the films were kept over night in an atmosphere of nitrogen in the presence of a piece of pyrogallol-potash paper subsequently drawn out by a thread, on adding carbon dioxide next day and exposing to light no trace of oxygen was found, although the films at the commencement of the exposure appeared to be green and normal. Apparently, therefore, the decomposition of chlorophyll on exposure to light and the resultant production of aldehyde is not accompanied by any appreciable evolution of free oxygen. Further, no hydroxyl could be detected in any of the films by testing with coloured cell sap and cyanin. Nor was the pink coloration produced on testing the films with decolorised magenta lessened by boiling the gelatine previously to testing, which should have been the case had any hydroxyl been present.

Dead cells of *Elodea* and *Vallisneria* were also tested by the bacterium methods for an evolution of oxygen, but without effect, using pure aerobic forms. If the green cells had been exposed to strong light for some hours until bleached and were then tested, it was found that the bacteria ceased to move or only moved very slowly in their immediate neighbourhood, even when the surrounding medium was fully supplied with oxygen. After the leaves had been boiled in dilute hydroxyl and soaked in cold water for a day, they showed the usual chemotropic attraction to aerobic bacteria in the presence of oxygen, due to the exudation of chemotropic substances. It is evident, therefore, that the aldehyde produced by the decomposition of the chlorophyll is able to prevent the detection of the presence of oxygen by the bacterium method, a danger which has not previously occurred to myself or to any previous worker with this method. This fact takes away all decisive value from the negative observations of Kny* and of Czapek† in regard to the absence of any power of evolving oxygen from isolated droplets of chlorophyll or from droplets imbedded in foreign protoplasm when tested by the bacterium method. The bacterium method must, therefore, be applied with great caution to chlorophyllous cells which have been exposed to strong light when in an abnormal, depressed, or dying condition. The only observations which seem to support those of Usher and Priestley, and show that an evolution of oxygen is possible when chlorophyll is exposed to sunlight apart from living protoplasm, are those of Molisch,‡ who found by the aid of luminous bacteria that chloroplastids from dried dead cells were able to evolve oxygen. Since, however, Molisch with the same means was unable under any circumstances to detect any evolution of oxygen from healthy cells rich in etiolin, but free from chlorophyll, it is evident that this method of Beyerinck's is not always reliable. The fact that chloroplastids may remain capable of photosynthesis outside the cell says nothing, for they do not retain this power long, whether kept in light or darkness, and have a photoplasmic basis. Nevertheless, it is quite possible that some of the cases of partial or complete assimilatory inhibition§ observed by me may result from an interference with the later processes of polymerisation, such interference rapidly reacting on the primary stages of photosynthesis. This is very probably the case when the accumulation of the products of photosynthesis increasingly retards further production. Photosynthesis is, however, certainly not the simple physico-chemical process that Usher and Priestley imagine it to be, but involves vital activity and control in all its stages.

It is, in fact, difficult to see how a simultaneous production of formaldehyde and hydroxyl could be possible in films exposed to sunlight, for as Geisou‖ has shown, hydroxyl reacts with neutral or acid solutions of formaldehyde, forming carbon dioxide, water, and hydrogen. This reaction is only shown in a test-tube on warming, but if a *Vallisneria* leaf which has accumulated formaldehyde is soaked in hydroxyl and exposed to strong sunlight or warmed, the formaldehyde disappears. Boiling with hydroxyl does not, however, remove from gelatine its power of producing a pink colour with decolorised magenta. Hence its "aldehyde" constituent is not formaldehyde, a fact which Dr. Rothero has confirmed by distillation tests.

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* Kny, 'Ber. d. Bot. Ges.,' 1897, vol. 15, p. 388.

† Czapek, 'Ber. d. Bot. Ges.,' 1902, vol. 20, p. (44).

‡ 'Bot. Zeit.,' 1904, vol. 62, I, p. 1.

§ 'Linn. Soc. Journ.,' 1896, vol. 31, pp. 429, 367.

‖ 'Ber. d. D. Chem. Ges.,' 1904, vol. 37, p. 515.

Usher and Priestley have, therefore, not founded the formaldehyde hypothesis upon any surer basis than had been already established for it by Pollacci* and by Curtius and Reinke,† the only established facts being that chlorophyll decomposes when exposed to light in the presence of oxygen and in the presence or absence of carbon dioxide, and that one of the products of its decomposition is formaldehyde. This production of formaldehyde does not, however, necessarily represent the primary stage in photosynthesis, but is either one of the later ones or a more or less accidental phenomenon shown either in abnormal or dead chlorophyllous cells and tissues or by extracted chlorophyll. In any case we have as yet no satisfactory proof that the production of formaldehyde in dead cells or extracted chlorophyll when exposed to light is accompanied either by a decomposition of carbon dioxide or by a production of oxygen or hydroxyl.

* 'Atti d. Instit. Bot. Pavia,' 1900, p. 45; 1902, p. 8; and 1904.

† 'Ber. d. Bot. Ges.,' 1897, vol. 15, p. 201.

On the Occurrence of Post-tetanic Tremor in Several Types of Muscle.

By David Fraser Harris, M.D., B.Sc. (Lond.), Lecturer on Physiology and Histology at the University of St. Andrews.

(Communicated by John G. McKendrick, M.D., F.R.S. Received February 15,—Read June 27, 1907.)

Part I.

In 1901* I discovered that a frog's gastrocnemius which had been kept in complete tetanus, either by direct or indirect stimulation, would, if the stimulation were continued until fatigue had begun to manifest itself, fall into a state of obvious tremor. This tremor, whose average periodicity in

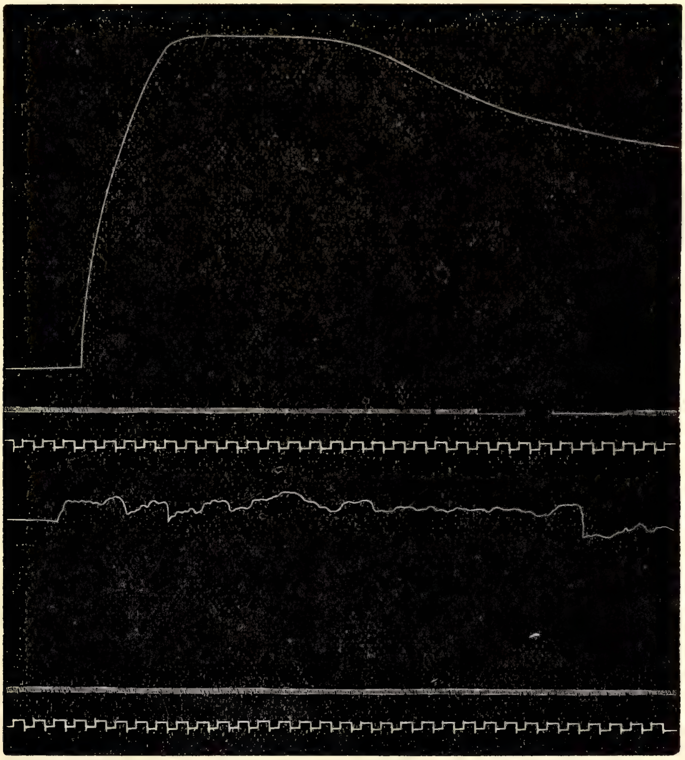

Fig. 1.—Gastrocnemius, frog. Indirect stimulation. 5 grammes close to axle of lever giving amplification of 14. Upper tracing, "complete tetanus" for about 25 seconds; lower tracing shows point of commencement of post-tetanic tremor. Periodicity at first 6 a second. Stimulation (Neef's hammer) is recorded above time in half-seconds. (Reduced to ½.)

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* D. F. Harris, 'Phys. Soc. Proc.,' March 1, 1903.

the frog's gastrocnemius is four to six a second, can be maintained unaltered as to its time-relations, but of slowly declining amplitude, for a relatively long time (half an hour).

Fig. 1 shows the smooth line of complete tetanus, breaking off into the post-tetanic tremor in the frog's gastrocnemius stimulated indirectly.*

On investigating as many animal types as I could procure, I found that a post-tetanic tremor could be demonstrated in man, cat, kitten, rabbit, pigeon, and frog, both with and without intact circulation, the mean periodicity of the tremor in all types being between two and six or eight per second.

An analogous tremor is elicitable in muscles of fish and lobster.

Fig. 3 is a facsimile of the tracing obtained by stimulating the human flexor sublimis digitorum by rapid induction-shocks.

After the time of complete tetanus is over, the muscle falls into a state of irregular tremor which in my own arm I caused to be maintained for half an hour, during which time it did not alter either in intensity or in average periodicity.

Sponge-electrodes were placed on two spots, an upper and a lower, on the fore-arm over the flexor sublimis digitorum. The middle finger had a ring slipped over its terminal phalanx, and from this a thread passed over a pulley to the spring of an ergograph.†

The interruptions here were 60 per second (30 makes and 30 breaks); and as there was previous independent evidence to show that the makes were subliminal, I may take it that 30 break shocks per second constituted the "stimuli."

The responses occurred with an average periodicity of four per second. The ratio of stimuli to responses is then 30 : 4 or 7·5 : 1, *i.e.*, 1 in 7·5 may be said to be the figure of physiological insusceptibility in this case. Obviously the muscle had its circulation intact.

[I may say that at the end of half an hour I experienced no sensation of fatigue in the muscle, but in the above and in all similar experiments on my own muscles the sensations of "the muscular sense" were quite distinct. A thrill corresponding to the rate of stimulation was perceptible.]

The next type of muscle I investigated was that in the cat. In one experiment, a young cat was pithed under a dose of A.C.E. mixture and artificial respiration done. The Tendo Achillis was fastened to a bell-crank lever, a 50-gramme weight being attached as near the axle as possible.

Direct stimulation of a muscle with intact circulation was kept up for half an hour, at the end of which time, while the muscle was still irritable, it was so feeble that a weight of 20 grammes had to be substituted. The average periodicity of the tremor is three to five per second.

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* The current in the primary circuit that was usually employed for tetanic stimulation was one of about 3·8 amperes (voltage 4·2).

† The "period" of this spring was 40 to 42 per second.

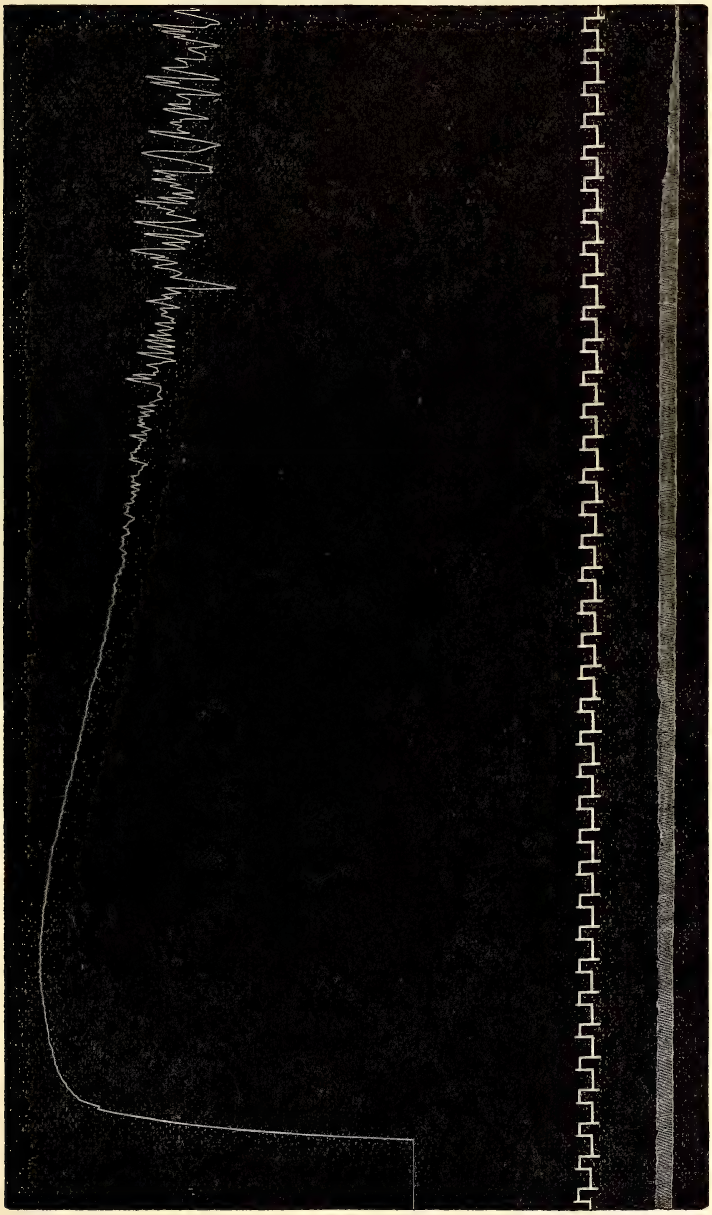

Fig. 2A.—Gastrocnemius, kitten. 10 grammes, same lever as in fig. 1. Stimuli from 100 a second tuning-fork in primary circuit, recorded below time in half-seconds. Onset of coarse tremor, 8 seconds from commencement of tetanus. Periodicity, 8; later, 5 to 6 a second. (With a lens a tremor can be seen during the complete tetanus, of periodicity 16 to 18 a second.)

In only one case, in mammalian muscle, did I obtain a rate slower than three to four per second, viz., one to two a second (fig. 4). Here the gastrocnemius was attached in the usual way to a bell-crank lever which carried a 10-gramme weight.

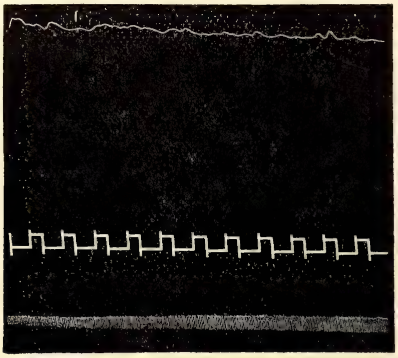

FIG. 2B.—Portion taken from middle of record of post-tetanic tremor several minutes after commencement. (Facsimile.)

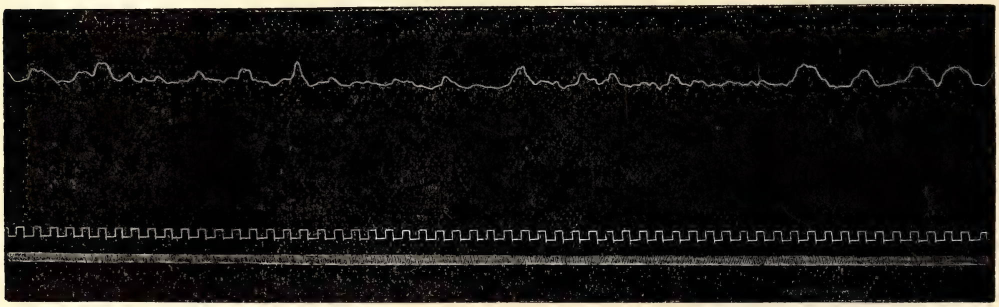

FIG. 3.—Flexor sublimis digitorum (homo); sponge-electrodes. Neef's hammer. Helmholtz side-wire in primary coil. Porter's ergograph-spring as recorder. Stimulation recorded below time in half-seconds. Tremor maintained for 30 minutes. Average periodicity 5 to 6 a second. (Facsimile.)

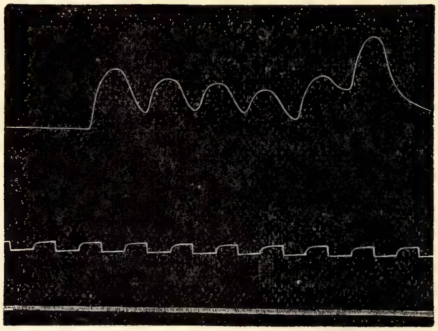

FIG. 4.—Gastrocnemius, kitten (pithed, artificial respiration). Complete tetanus (on direct stimulation) suddenly breaking off into post-tetanic tremor.

The animal was pithed, artificial respiration was used, and very little blood lost in preparing the muscle. With indirect stimulation, the muscle exhibited complete tetanus for an unusually long time; several minutes after the stimulation had been made direct (owing to the onset of end-plate fatigue) the tetanus was still complete, but after a few seconds more the muscle quite suddenly broke off into a tremor with a periodicity of about one or two per

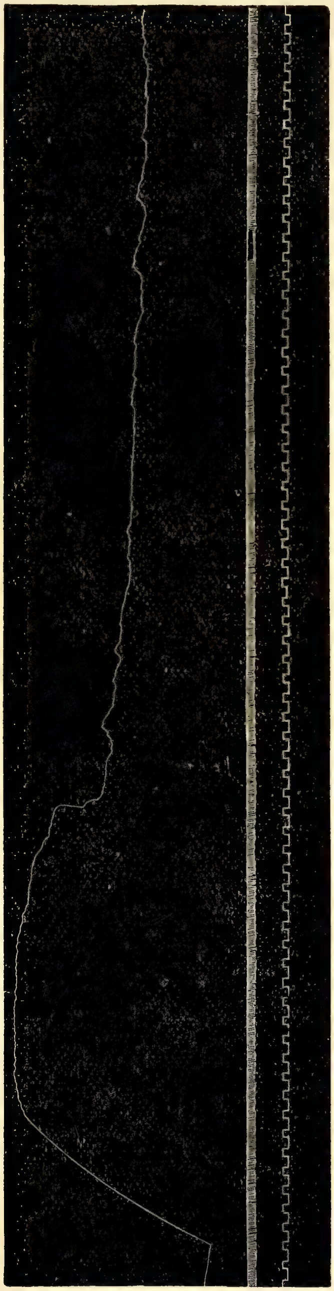

Fig. 5.—Extensor longus digitorum, pigeon, pithed. 20 grammes near axle. Lever as in fig. 1. Stimulation direct. Stimulation by Neef's hammer (side-wire), recorded above time in half-seconds. Tremor recorded for 20 minutes. Periodicity, 3 to 5 a second.

second which, becoming rhythmic, was maintained of singularly constant intensity.

To investigate the post-tetanic tremor in the bird, I used the pigeon (pithed). The extensor of the toes was the muscle chosen: a cord from the tendon for the toe passed to a hinged lever weighted with 20 grammes quite close to the axle. Direct stimulation was maintained for 20 minutes; portions of the tremor during this time are reproduced in fig. 5. The average periodicity of the tremor is three to five a second.

In the case of the muscles of the fish, in some experiments I used those of the tail of the plaice or "spotted flounder" (*Pleuronectes platessa*), the fish being pithed. Considerable difficulty was encountered in fixing the very soft-fibred muscles to the recorder.

Using the hammer as interruptor, and direct stimulation, I obtained a tremor of about one per second, which could not, however, be maintained indefinitely; fatigue seemed to supervene with considerable rapidity; I found great difficulty in bringing out smooth complete tetanus at all: the tendency to tremor was present from the first.

When we used the muscles that close the jaw we obtained a tremor of a very slow periodicity, a shortening lasting about 1 second and occurring at irregular intervals of 3, 4, 5, or 6 seconds each.

In the case of the lobster (*Homarus vulgaris*), we used one of the flexors of the abdominal somites. The preparation was fixed by clamping a portion of the carapace in muscle-forceps and attaching the fibres of the muscle to a horizontal lever with a weight (10 grammes) very near the axle. The stimulation was, of course, in all cases direct, fine wires being led from the short-circuiting key in the secondary circuit directly into the muscle substance.

On submitting the muscle of the lobster to stimulation similar to that employed in most of the other types of muscle (viz., stimuli given by Neef's hammer, with side-wire), I failed entirely to produce complete tetanus; in other words, the muscle fell into a state of tremor from the very beginning of the stimulation.

This is very well seen in fig. 7, where, using a quite fresh flexor of abdominal somite, a violent tremor is elicited without any preliminary period of complete tetanus.

The periodicity of this tremor in the first second was nine a second, but, as fatigue set in, it fell to three a second.

Thus, although we cannot use the term "post-tetanic" of the tremor which appears in the muscle of the fish or of the lobster in consequence of stimulation by rapidly recurring (tetanic) stimuli (30 to 70 a second), never-

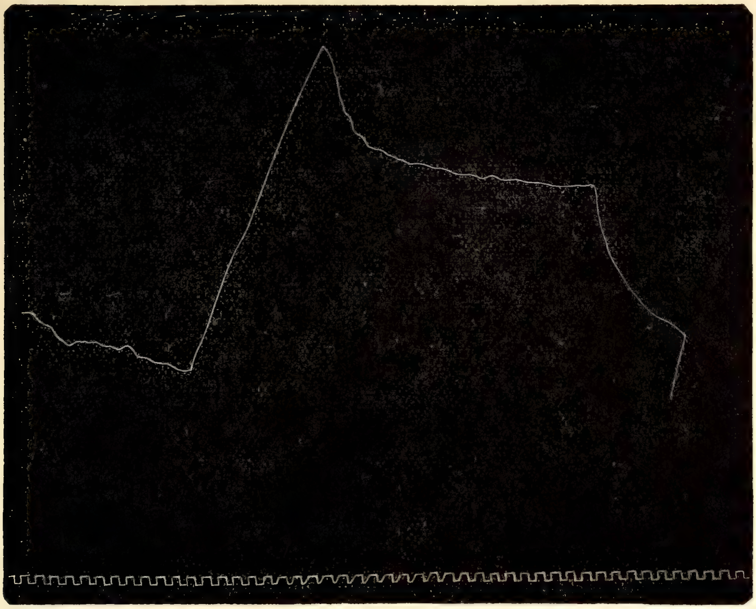

FIG. 6.—Tail-muscle of *Pleuronectes platessa*. 5 grammes near axle. Lever as in fig. 1. Neef's hammer, side-wire. Stimulation direct. Time in half-seconds. No complete tetanus. Tremor of periodicity, 1·5 to 2 a second.

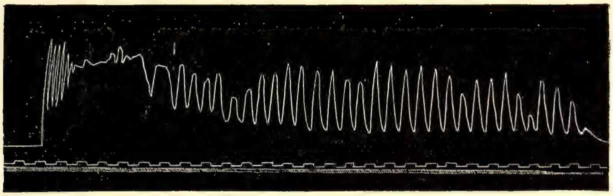

FIG. 7.—Muscle of abdominal somite, *Homarus vulgaris*. Lever as in fig. 1. Stimulation direct. Break-shocks only. 10 grammes near axle. Stimulation, given by Neef's hammer (side-wire), recorded below time in half-seconds. Tremor from commencement. Periodicity at first 9 a second, soon becoming 3 a second. (Tracing reduced to one-half.)

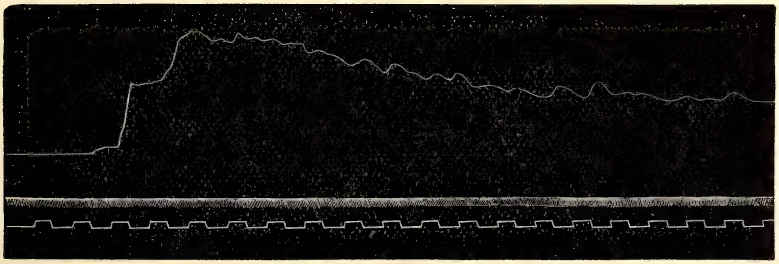

FIG. 8.—Tremor in a not quite so fresh muscle of lobster, average periodicity 4 to 5 a second. Stimuli at least 70 a second recorded above the time in half-seconds. Conditions as in fig. 7. (Facsimile.)

theless the time-relations of this tremor are comparable with those of the post-tetanic tremor of the higher muscular types.

Crustacean muscle is no exception to the universality of this phenomenon of slow tremor appearing in muscles subjected to "tetanising" stimuli; it possesses, in common with mammalian, avian, and amphibian muscular protoplasm, that property of functional insusceptibility in virtue of which it avoids exhaustion by falling into a state of comparatively slow "rhythmic" contractions.

PART II.—ANALYSIS OF RESULTS AND THEORETICAL.

(1) The first point that may be noticed here is that the tremor of post-tetanic onset is in its average periodicity *not* that of the tremor of the same muscle about to pass from incomplete to complete tetanus. *E.g.*, in frog's gastrocnemius the maximal rhythm of the incomplete tetanus (just before fusion to complete) is something between 25 and 28 per second,* while the "rhythm" of the tremor that follows spontaneously on a period of complete tetanus is about four to six a second. Instead of 25 to 28 responses per second, the muscle can now exhibit no more than five to six, or about one-fifth of the former.

Obviously, fatigue is the cause of this physiological insusceptibility; fatigue which, by preventing responses at anything like the rhythm of the stimuli (30 to 100 per second), prevents the early onset of complete *exhaustion.* This physiological insusceptibility may be taken as a protective mechanism against the fatal effects of full fatigue.

(2) The post-tetanic tremor is a myogenic phenomenon. It occurs in muscle directly stimulated, whether curarised or not, and a tremor indistinguishable from it is given by, *e.g.*, the dying diaphragm (fig. 10). For tremors of directly stimulated muscles, see figs. 2B and 3. For tremor of curarised muscle (toad), see fig. 9.

The so-called "spontaneous" tremor I was able to record for three quarters of an hour in the dying diaphragm of a pithed rabbit which had its phrenics cut. It is a tremor of about six per second average periodicity and, therefore, quite similar in time-relations to the post-tetanic.†

(3) While the periodicity of the post-tetanic tremor is rarely, during long periods, more than six per second, it is not of the same periodicity in all the muscles even of the same animal.

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* T. G. Brodie, 'Elements of Experimental Physiology,' pp. 62, 63. London: Longmans, Green, and Co., 1898.

† D. F. Harris, "On the Time-relations of the Spontaneous Tremor of the Diaphragm," 'Phys. Soc. Proc.,' January 26, 1907.

Thus, while in the frog's gastrocnemius it is about six per second, in the hyoglossus it is only 2 to 2·5 a second; ratio 3 : 1.

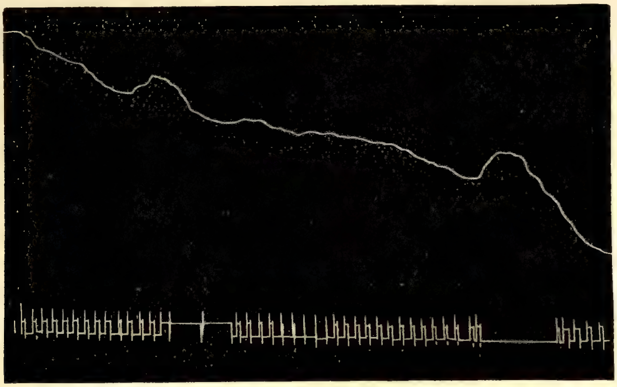

Fig. 9.—Gastrocnemius, toad, curarised. 5 grammes. Bell-crank lever. Stimuli by Neef's hammer (side-wire). Time in half-seconds. Tremor maintained for 15 minutes. Average periodicity, 2·5 to 3 a second. (Facsimile.)

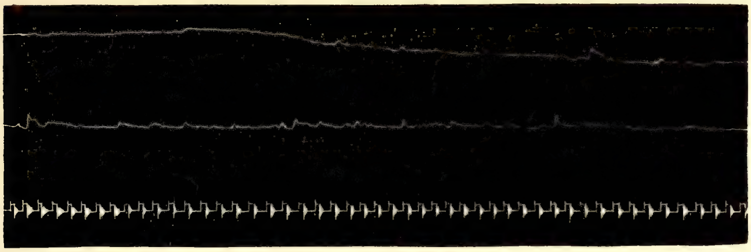

Fig. 10.—Diaphragm, rabbit (pithed, phrenics cut). "Spontaneous" tremor recorded for $\frac{3}{4}$ hour. Time in half-seconds. Lever as in fig. 1; no weight. Periodicity at first 10 a second; later, 5 a second. (Facsimile.)

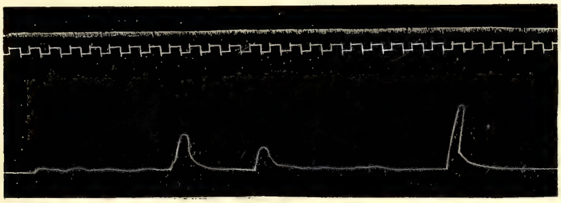

Fig. 11.—Hyoglossus, frog. Direct stimulation; no weight. Neef's hammer (side-wire). Time in half-seconds. Tremor. Periodicity, 2 to 3 a second.

This difference is interesting in the light of the well-known difference in the number of stimuli necessary to cause complete tetanus in the gastrocnemius and in the hyoglossus respectively, viz., 30 and 10 respectively, ratio 3 : 1.

The post-tetanic tremor, no less than the genesis of their tetanus, proclaims the differences in molecular mobility between the gastrocnemius and the hyoglossus muscles.

Similarly, a poison like curare, which diminishes protoplasmic mobility, brings down the periodicity of the post-tetanic tremor from the six per second of frog's normal gastrocnemius to 2·5 to 3 per second in the curarised gastrocnemius (*cf.* fig. 9).

In other words, the physiological insusceptibility is now about three times as great as in the normal frog muscle; or, again, the curarised gastrocnemius becomes reduced, as regards molecular mobility, to the level of the less mobile hyoglossus.

(4) The post-tetanic tremor is characteristically irregular in intensity from moment to moment.

Previously to my publishing the first note on the periodicity of this tremor, Dr. Bayliss was good enough to suggest, in a private communication, that the irregularity in the record of the tremor was due to irregular variations in the intensity of the various members of the series of stimuli given by Neef's hammer, that is to say, that the stimuli varied from submaximal to maximal and *vice versâ*. I had eliminated this possibility, by noticing that post-tetanic tremor could be produced at all distances between the primary and secondary coils, both when the stimuli were submaximal and when they were maximal.

As I had already used the Helmholtz side-wire equaliser, and did not consider that the vibrating reed gave stimuli of sufficiently uniform intensity, I decided to test the point by (1) using a 100 a second electro-magnetic tuning-fork as interruptor in the primary, and (2) by using as interruptor, in the primary circuit, a metallic rotating wheel provided with teeth which dipped into a pool of mercury, and was driven at varying speeds by a small air-engine.

By neither of these methods was the irregularity abolished; and in the case of certain muscles was not in any obvious manner diminished (notably fig. 2, muscle of kitten, where the 100 a second fork was used).

While freely admitting it possible that a series of stimuli of absolutely equal physical intensity would give the least irregular form of post-tetanic tremor, yet I am compelled, from a study of a large number of these tremors through long periods during which the stimuli were as constant in energy as possible, to believe that the irregularity of the post-tetanic tremor is inherently characteristic of it.

It seems to me that the irregularities can be explained by:—

1. Non-simultaneous onset of fatigue in the several fasciculi or fibres of the muscle; so that when we use even physically equal stimuli, a stimulus which is effective for one fibre or fasciculus may not be so for another, or if effective

for one fibre, etc., at one moment, may not be so for that same fibre at the next moment.

All the fibres are not equally fatigued after the same duration of stimulation, and, through their irresponsiveness, certain fatigued fibres rest, to recover their irritability later on, so that a stimulus sub-liminal for a given fibre at one moment may be liminal or supra-liminal for it after a short interval.

2. In the next place, it is not probable that all the fibres composing the depths of a muscular mass receive either in indirect or in direct stimulation their stimuli at absolutely the same instant through the entire muscle—the so-called "non-instantaneous innervation;" and, further, it is known that certain fasiculi have more component fibres than others, more fibres to be innervated.

It rarely happens that all the fibres contract simultaneously, but when they do, we obtain a result as in fig. 4—a series of large waves of quite slow rhythm, 1·5 to 2 a second—reminding one strongly in some respects of a cardiac rhythm as seen in fig. 12, which is a record of the so-called "heat-tetanus" of the ventricle of the heart of a rabbit.

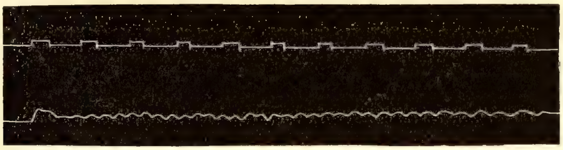

FIG. 12.—Ventricle of heart (rabbit), immersed in continuously heated 1-per-cent. NaCl. Lever same as in fig. 1. 5 grammes weight. Time in half-seconds. Maximal periodicity, 6 to 7 per second.

In this case the gastrocnemius muscle had its circulation intact (kitten pithed, artificial respiration); the stimuli were from a tuning-fork in the primary, *i.e.*, as physically uniform as possible; stimulation was direct. Here I conclude that the onset of fatigue was simultaneous throughout, practically, all the fibres of the muscle, a condition, for several reasons, very rarely met with (fig. 4).

Here, if anywhere, the conditions were such as to favour the simultaneous onset of fatigue in the fibres, for the stimuli were as equal in intensity as was possible, and any conditions due to varying degrees of neural fatigue were eliminated by the previous fatigue of the motor end-plates; all the fibres were still supplied with oxygenated blood, *i.e.*, were under the same conditions as regards nourishment and removal of waste-products, so that there was maximum uniformity of both the physical and metabolic conditions.

Except for a second or two, I have seen none but irregular tracings of post-tetanic tremors, and this irregularity also characterises even those tremors which are produced by the so-called "constant" stimuli and also certain tremors of "spontaneous" origin.

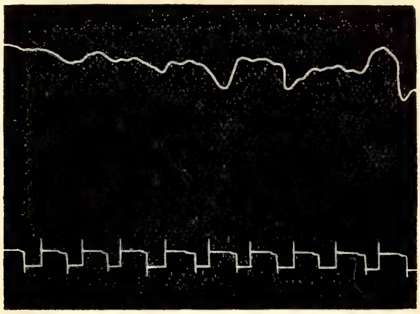

Fig. 13.—Gastrocnemius, frog. Sciatic nerve drying. Bell-crank lever; no weight. Time in half-seconds. Periodicity of tremor, 4 to 6 a second. (Facsimile.)

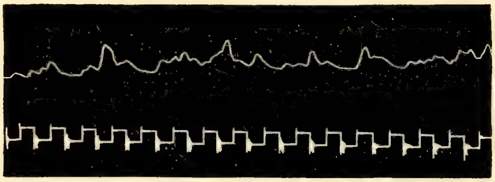

Fig. 14.—Gastrocnemius, frog. Solid NaCl placed on sciatic nerve. Bell-crank lever; no weight. Time in half-seconds. Periodicity of tremor, 5 to 7 a second. (Facsimile.)

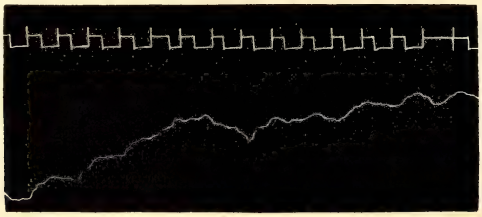

Fig. 15.—Gastrocnemius, frog. Sciatic nerve pinched. Bell-crank lever; no weight. Time in half-seconds. Periodicity of tremor, 6 a second. (Facsimile.)

With regard to tremors due to "constant" or "single" stimuli, we have the tremors from (1) drying of the nerve; (2) chemical stimulation of the nerve, *e.g.*, by NaCl; (3) pinching the nerve; (4) heat suddenly applied to the nerve; and (5) the disappearance of anelectrotonus from the nerve: while the tremor of the dying diaphragm is an example of the "spontaneous" kind (*cf.* fig. 10).

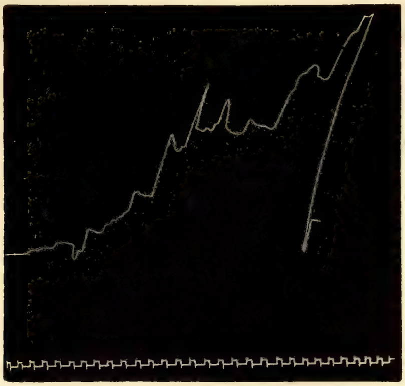

Fig. 16.—Gastrocnemius, frog. Sciatic nerve suddenly heated by hot NaCl 75-per-cent. solution. Bell-crank lever. Time in half-seconds. Periodicity of tremor, 4 per second. (Facsimile.)

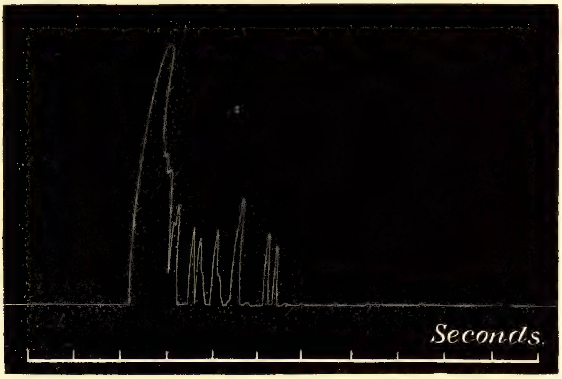

Fig. 17.—Gastrocnemius, frog. Ritter's "opening tetanus." Bell-crank lever; no weight. 1 cm. of sciatic nerve had "constant" current ($5{\cdot}4 \times 10^{-5}$ ampere) ascending for 5 minutes through non-polarisable electrodes. To be read from left to right. Time in seconds. Periodicity at beginning of tremor, 5 to 6 a second. (Facsimile.)

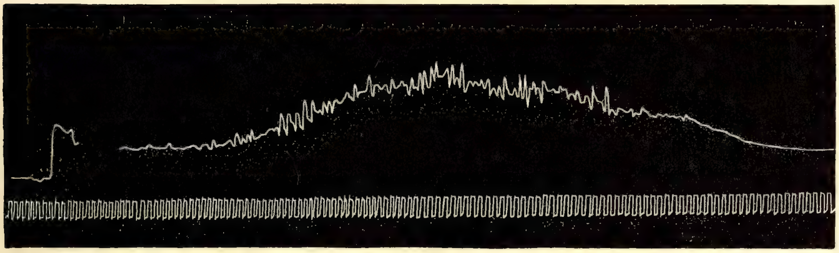

Fig. 18.—Sartorius, frog, completely immersed in Biedermann's fluid. To be read from left to right. Time in half-seconds. Average periodicity, 4 per second. (Facsimile.)

All of these tremors are more or less irregular.

Now all these tremors are, as to average periodicity, of the same order as the post-tetanic (three or four to six a second), and as the coarse tremor brought on by fatigue in, *e.g.*, the human deltoid (fig. 19).

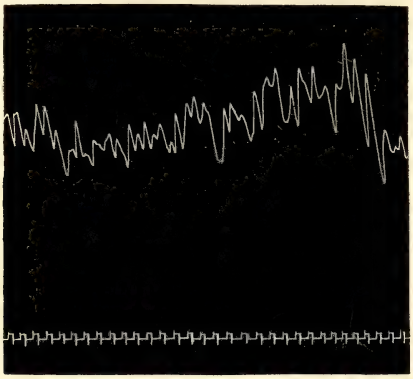

Fig. 19.—Deltoid, human, coarse fatigue tremor of. Arm held out horizontally with 1 kilo. in hand; cord from middle finger to button of cardiograph (Marey's). Tracing by a recording tambour. Time in half-seconds. Periodicity of tremor, 5 per second. To be read from left to right. (Facsimile.)

In these cases we may say that *inco-ordination* of fibres or of fasciculi is the element common to all, whether this has been brought about by "non-simultaneous innervation" or by repeatedly varying degrees of the excitability of the fibres brought on either by fatigue or by some hitherto unexplained state of inaccessibility to certain stimuli.

I take it that this inco-ordination or non-simultaneousness of innervation is what Dr. Haycraft alludes to when, discussing normal voluntary tetanus, he writes . . . "muscles exhibit fascicular or other *local* movements."*

If this be true as regards normal voluntary tetanus, the record of which is very regular compared with the tremors under study at present, it must apply with increased force to those tremors of muscles in which fatigue has set in.

While fatigue, inducing fascicular inco-ordination, can be held to explain the irregularity of the post-tetanic tremor and the spontaneous tremors of dying muscle, it is difficult to see how fatigue can be the main factor producing the irregularity of such tremors as those from chemical, mechanical, or thermal stimulation or from anelectrotonus, seeing that the irregular contractions are present from the very first when fatigue obviously cannot have set in.

The probability is that those stimuli called "single" or "constant" are, as regards nerve-molecules, anything but constant in their stimulating power.

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* J. B. Haycraft, "Voluntary and Reflex Muscular Contraction," 'Phys. Journ.,' vol. 11, 1890, p. 366.

Drying of nerve in air, abstraction of water by NaCl, increase of temperature, even pinching the nerve cannot be assumed to be respectively uniform in their modes of being stimuli; in fact, physically they represent stimuli varying considerably in intensity from moment to moment. They introduce intra-molecular disturbances of various potentials and of varying disruptive powers as regards the biogens of the nerve. These disturbances "act" like discrete stimuli of varying intensities.

Theoretical Explanation of the Meaning of the Post-tetanic Tremor and Tremors of Similar Periodicity.*

The property of functional inertia of muscular protoplasm expressed here as a physiological insusceptibility to certain stimuli seems to me to furnish the key to the meaning of the post-tetanic tremor. I regard it as a protective mechanism which, by permitting the establishment of this fatigue-rhythm of low periodicity, averts for a time the physiological calamity of full exhaustion.

If there *could* be responses to such high rates of stimulation as 30, 40, or 100 per second, the muscle would very soon be utterly tired out and incapacitated for a long time from activity. But, owing to the possession of the property of physiological irresponsiveness (functional inertia), the muscle subjected to continued stimulation responds only to some of the stimuli in the rapid series (every fifth, seventh, or tenth), or responds at a rhythm very much lower than that of the stimuli, thus substituting chronic fatigue for acute exhaustion.

Here, in virtue of functional inertia, the living matter preserves itself from that destruction which would overtake it were it possessed of affectability alone. The two properties are co-existent in the living matter: resting muscle has much affectability, but little functional inertia; as activity proceeds, the affectability diminishes and the functional inertia increases towards the continued stimulation, until a point is at last reached when the ratio between the two properties is such that only every 1 in 7 or 10 stimuli is responded to, the others being functionally disregarded. This state of matters can be exhibited for a long time, biologically speaking, viz., half an hour, as in the post-tetanic tremor of the human flexor sublimis digitorum with intact circulation.

The biotonic state at any moment depends on the ratio of the degrees of possession of these two properties; were affectability alone present we would have, as a result of perfect response to all stimuli, a maximum uncontrollable activity, and the rapid end of things in death; were functional inertia alone present, we would have complete irresponsiveness to all stimuli.

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* D. F. Harris, "Functional Inertia, a Property of Protoplasm," 'Roy. Soc. Edin. Proc.,' vol. 24, p. 196; and D. F. Harris, "Affectability and Functional Inertia as *the* two Properties of Protoplasm," 'Scot. Micros. Soc. Proc.,' vol. 4.

But a condition of functional compromise between the two biotonic extremes is set up, and thus a mean condition, known as "fatigue," established. I take the existence of the post-tetanic tremor as one more expression of the physiological significance of fatigue and of rhythm.

In those tremors arising from a single stimulus (figs. 15, 16, and 17), or from continued stimulation (figs. 13 and 14), or spontaneously (fig. 10), we have the property of functional inertia of the muscular substance contributing to the explanation of the feature common to them all, the *want* of correspondence between the character of the responses and the nature and mode of application of the stimuli.

Tremors of the same average periodicity, 3 to 6 a second, are elicited by stimuli physico-chemically the most varied, and may be seen characterising cases so far apart physiologically as the dying diaphragm and the maximal rate of beat of the heart ventricle.

This non-correspondence or lack of parallelism between stimulation and response seems to be due to the stimulus-disregarding property of functional inertia in the muscle-substance, the same property which, in the case of long-continued rhythmic stimulation, expresses itself in the post-tetanic tremor.

Through affectability muscle responds to any stimulus by contracting, but through functional inertia the nature, the mode of application, and even the rhythm of the stimulation is *disregarded,* and to the most varied kinds of stimulation the response becomes the same—a tremor whose component events recur with the same slow average periodicity.

"It seems," writes Professor Biedermann,* "to be an almost universal property of muscular substance to fall, under certain conditions, with all prolonged stimuli into a state of visible rhythmical excitation." The above data, I submit, are an experimental and statistical verification of this statement.

[The expenses involved in the foregoing work have been met by a grant from the Carnegie Trust for the Universities of Scotland, of which I here desire to express my grateful acknowledgment.]

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* 'Electro-physiology,' vol. 1, p. 107. London : Macmillan, 1896.

On Reciprocal Innervation of Antagonistic Muscles. Eleventh Note.—Further Observations on Successive Induction.

By C. S. Sherrington, D.Sc., F.R.S.

(Received November 4,—Read December 5, 1907.)

(Physiology Laboratory, University of Liverpool.)

It was shown in previous notes* of this series that in the "flexion-reflex" of the limb the reflex inhibition of the extensor muscles is frequently followed by contraction of them. This contraction ensues immediately on cessation of the inhibitory stimulus, and is often more accentuated than was the contraction originally inhibited. The phenomenon was ascribed to a process of rebound in the central part of the nervous arc inhibited, and this process, on account of its seeming analogy with certain visual phenomena often called "inductive," was termed "successive induction."

The occurrence of this "successive induction" made it seem probable that under favourable conditions the reflex evoked in the limb by a single stimulus would be diphasic with a first phase of flexion followed by a subsequent phase of extension. The following observations show that this is in fact the case, and under appropriate conditions regularly so.

Among conditions requisite for the reflex's exhibition of this diphasic character is freedom of the preparation as far as possible from such depressing influences as "shock," deep narcosis, fatigue, etc. In purely spinal reflexes successive induction does occur; thus, it causes exaltation of activity of the extensor arcs to ensue after their inhibition during a flexion reflex; that was established by observations previously reported.† In those observations the reflex preparation used had been preserved over long periods subsequent to spinal transection, the depression due to spinal shock in the ordinary meaning of the term had had prolonged opportunity to subside. In the present observations the examination of the reflex has been proceeded to within a few hours of the transection, since it was found that the successive induction could be observed without recourse to long periods of recovery if the transection instead of being spinal was pontine, *i.e.*, through the anterior part of the hindbrain. The transection and

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* 'Roy. Soc. Proc.,' *B*, vol. 76, p. 160; vol. 77, p. 478; vol. 79, p. 347; also 'Integrative Action of the Nervous System,' London and New York, pp. 21, 206, 1906.

† Sherrington, 'Roy. Soc. Proc.,' B, vol. 76, p. 161; vol. 77, p. 478; 'Integrative Action of the Nervous System,' p. 19; also article "Spinal Cord," Schäfer's 'Text-book of Physiology,' vol. 2, p. 841, 1900.

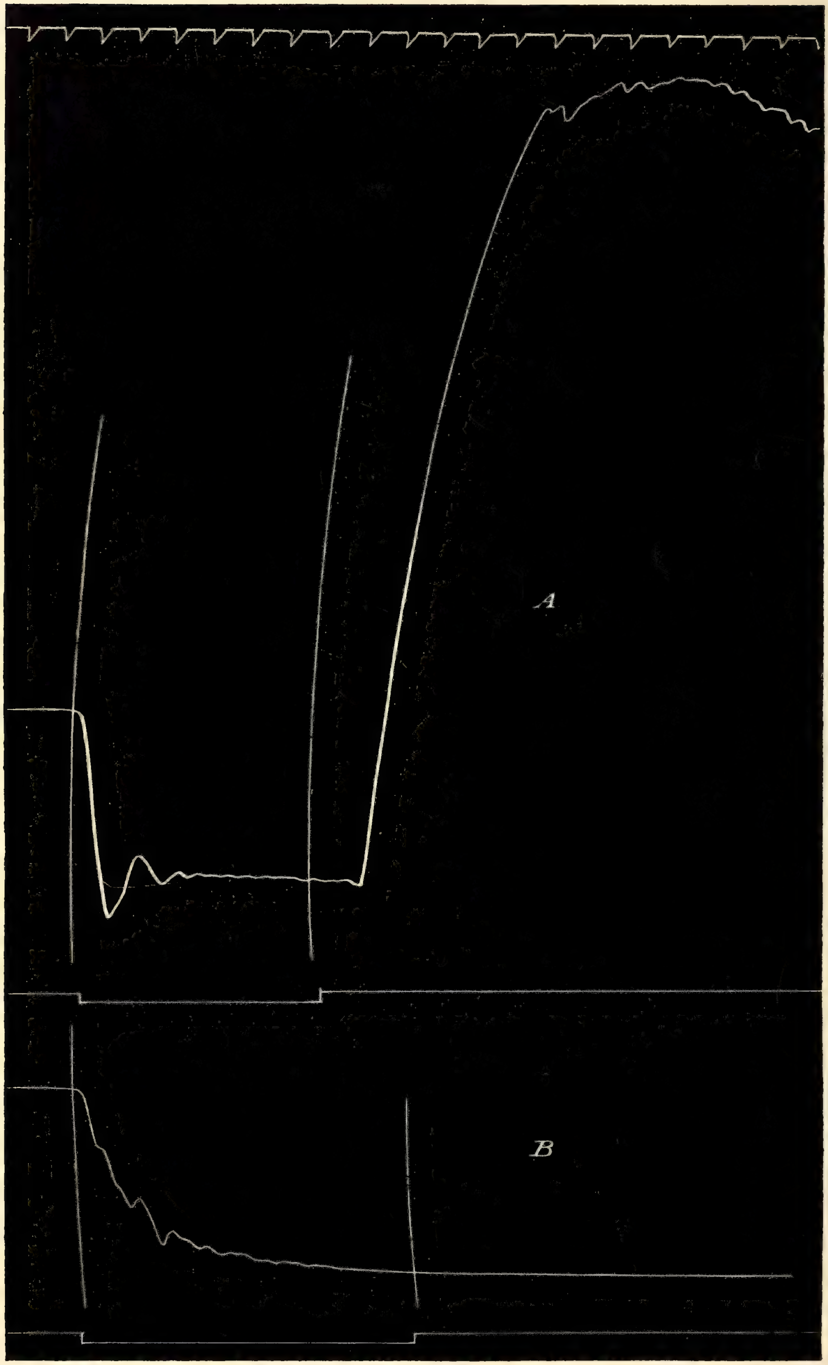

FIG. 1.

operation preparatory to that was carried out under deep chloroform narcosis. The brain anterior to the transection was removed. After this removal the chloroform narcosis was lightened. The "flexion-reflex," elicited from an afferent nerve or from some appropriate skin-point, is then found to be diphasic. The movement of active flexion is followed by a movement of active extension. During the active flexion the extensor muscles are relaxed by central inhibition; during the active extension the extensor muscles contract.

The contraction of the extensor muscle, *i.e.*, the discharge of the extensor motoneurones, never in my experience sets in during the actual delivery of the external stimulus used to excite the "flexion-reflex." So long as that stimulus continues the extensors remain inhibited (fig. 1).* It is only after withdrawal of that stimulus that the discharge of the extensor motoneurones occurs. The commencement of this discharge, judged by the muscle's contraction, follows withdrawal of the external stimulus by a time interval longer than that between commencement of external stimulus and onset of the inhibitory relaxation of the muscle. In other words, the latency of the contraction-phase is longer than that of the inhibition-phase (fig. 1, A, and fig. 8, A).

That the contraction of extensor muscle, *i.e.*, discharge of extensor motoneurone, never occurs *during* the continuance of the external stimulus, distinguishes the reaction from a rhythmic reflex such as the "scratch-reflex." In the scratch-reflex the phases of absence of discharge and of occurrence of discharge succeed each on the other in the motoneurone, *e.g.*, of extensor of knee, during unabated continuance of the external stimulus,† and with a period of alternation practically independent of the external stimulus. In the case of the "flexion-reflex" of diphasic character, the second phase, the phase of discharge of the extensor motoneurone, cannot apparently break through the state of inhibition characterising the first phase so long as the external stimulus is unremitted. The first phase may be termed, if desired, a refractory phase; but if so it is a refractory phase the duration of which depends on the external stimulus, whereas the duration of the refractory phase of the scratch-reflex is independent of the external stimulus.

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* The time-marker records in fifths of seconds throughout all the figures. The signal marking excitation is below. All read from left to right. In all the records (figs. 1—9 inclusive), descent of the myograph line means relaxation of the muscle, and conversely its ascent means contraction of the muscle.

† Sherrington, 'Journ. of Physiology,' vol. 29, p. 58, 1903; vol. 31, p. xvii; vol. 34, p. 1; 'Integrative Action of the Nervous System,' p. 45.

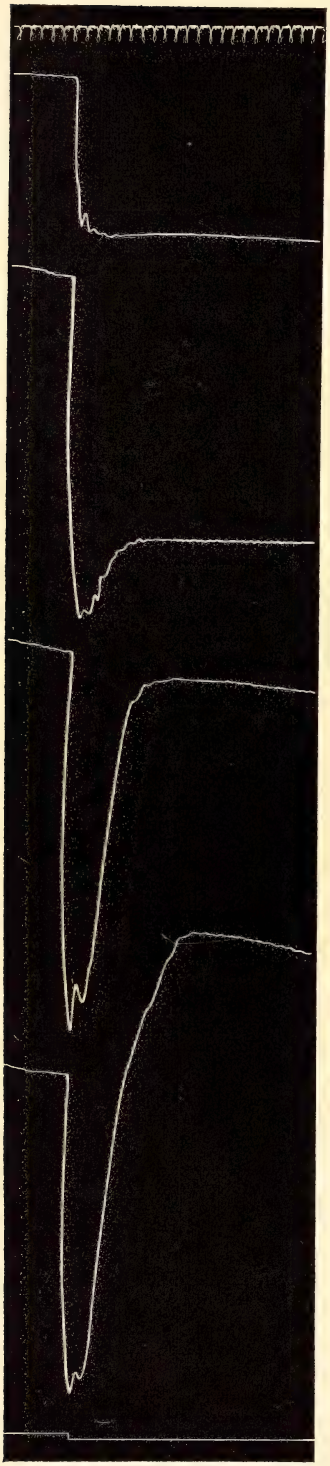

FIG. 2.

For the contraction-phase to ensue, not only must the external stimulus have ceased, but that stimulus must have possessed an intensity of above a certain minimal degree.

In fig. 1, the lower reaction B, showing no after-phase of contraction, was given by a weaker repetition of the faradic stimulus which in the same reflex preparation had shortly before caused the upper reaction A, showing marked after-phase of contraction. This influence of intensity is well shown when the stimulus is very brief, *i.e.*, a single make or break induction shock. When the shock is a weak one, inhibitory relaxation of the muscle is its only effect obvious in the preparation (fig. 2). When the shock is more intense, a phase of contraction follows the phase of relaxation (fig 2), and the contraction-phase becomes more marked as the intensity of the shock is made greater (fig. 2). With a quite strong induction shock, *e.g.*, a break shock unpleasant to the observer's tongue, the contraction-phase ensuing on the relaxation may reach a height much exceeding that of the contraction pre-existent to the inhibitory relaxation (fig. 2). Also, the contraction-phase may last several seconds, and be much longer than the phase of inhibitory relaxation which preceded it (fig. 2).

With longer and more complex stimuli, such as series of double shocks at an intermittence of 30 per second, a similar relaxation is even better evident (fig. 3). When the stimulus is quite weak, the reflex effect in the extensor muscle, *e.g.*, *vasto crureus*, is simply an inhibitory relaxation (fig. 3, A). As the stimulus is repeated with greater intensity, a phase of contraction follows on the phase of inhibition, and the contraction-phase increases with increase of the intensity of the stimulus (fig. 3, B, C, D). But the phase of contraction never encroaches on the phase of inhibitory relaxation during the period of actual delivery of the external stimulus.

Further, with these stimuli other conditions governing the appearance and intensity of the contraction-phase become apparent. Of these, one is that a

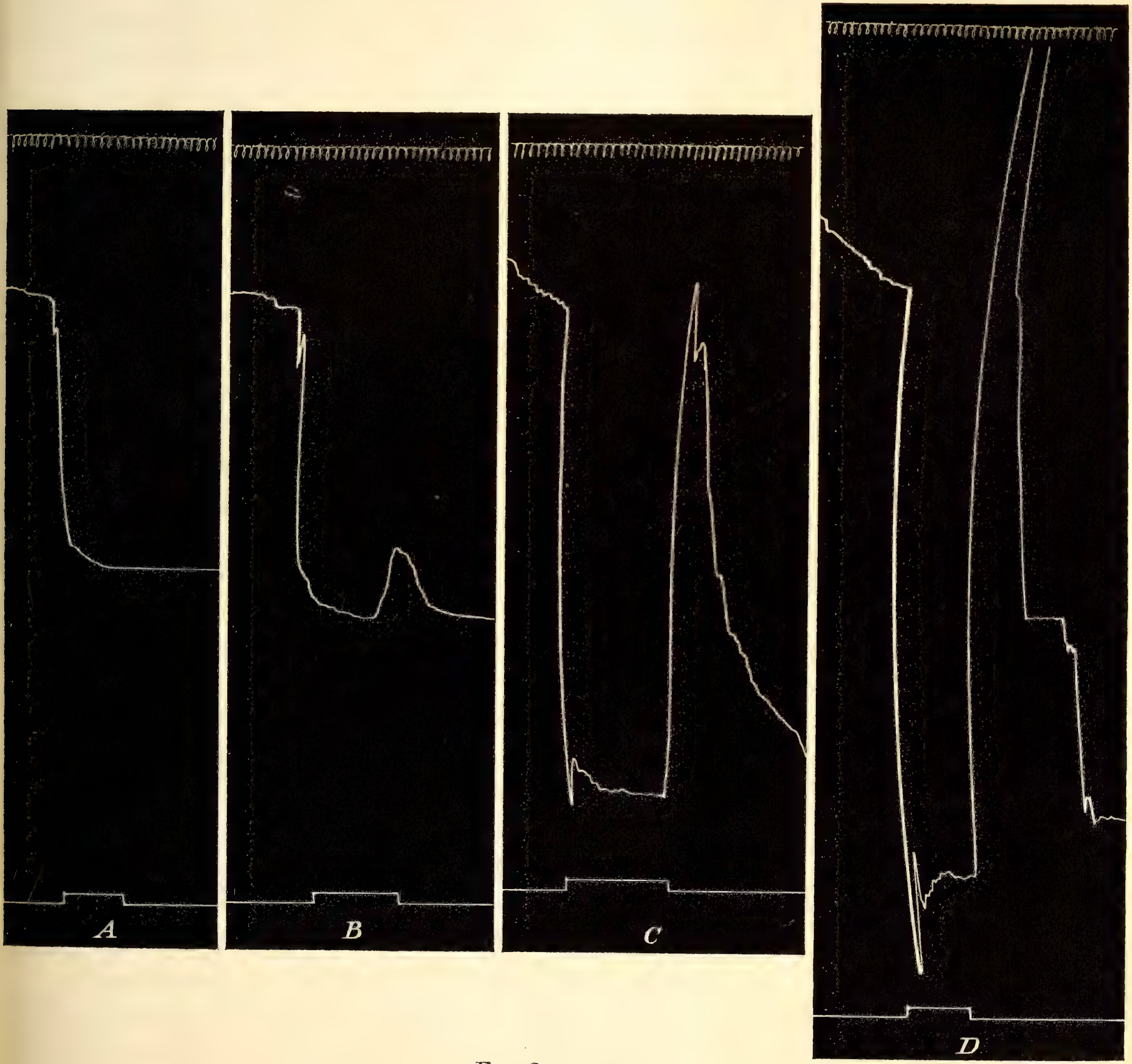

Fig. 3.

weak stimulus which, when brief, does not occasion a contraction-phase, does induce a contraction-phase if its duration be lengthened (fig. 4). Also, a stimulus which, when brief, occasions but slight contraction-phase, occasions more and more marked contraction-phase when its duration is more and more lengthened, up to certain limits. That it is less easy to evoke the diphasic reflex by a single induction shock as stimulus than by a faradic stimulus, is explicable by the influence of mere duration of the stimulus upon the efficacy of a stimulus to induce the contraction-phase. The rule holds generally in reflex reactions that frequently-repeated small stimuli sum to a total effect equal to that of a single stimulus of much greater individual intensity. The above results amount, therefore, simply to this, that the phase of inhibition has to exceed a certain minimal value in order that a phase of contraction ensue from it.

In fig. 3 it will be noticed that the amount of lengthening of the muscle in the inhibitory phase of the reflex increases with the strength of the stimulus, as does the ensuing contraction-phase itself. Yet it is not at all necessary, in order to obtain the contraction-phase, or to obtain a marked contraction-phase, that the lengthening of the muscle in the precurrent inhibition-phase should have been extensive. The intensity of contraction-phase bears no close relation to the amplitude of the lengthening in the inhibitory phase. The amount of contraction-phase is independent in great measure of the extent of the fore-running inhibitory lengthening of the muscle. Thus, in figs. 4, 5, 6, and 7, the amplitude of the lengthening registered in the inhibition-phase was very small, yet the ensuing contraction-phase is marked in fig. 4, and is very great in figs. 5 and 6. The amplitude of the lengthening of the muscle, which takes place during the inhibition-phase, depends much on the degree of contraction present at the moment when the inhibitory stimulus is applied. If the muscle be then already fairly at relaxation length, the amount of further lengthening which ensues during the inhibition-phase may be small. The stimulus will then, nevertheless, if it have sufficient intensity or duration, be followed by large contraction-phase (figs. 5, 6, and 7). This seems important, because it indicates that the main predisposing factor for the after-coming contraction is not the peripheral relaxation of the muscle, but the central process of inhibition, and this the muscle only fully expresses under suitable mechanical conditions. We may suppose that the central change underlying this central inhibition increases with increasing intensity of stimulus—intensity including, as was argued above, duration (within limits) of stimulus. The amount of the after-coming contraction-phase seems, therefore, proportioned to the amount of the precurrent inhibition which takes place *centrally*. This argues in favour of regarding the contraction-phase as due to a central rebound from inhibition to excitation.*

It was said above that a stimulus, too weak when brief to evoke an after-phase of contraction, becomes effective when prolonged (fig. 4). The range of

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* *Cf.* "Integrative Action, &c.," p. 212; 'Journ. of Physiology,' vol. 36, p. 191.

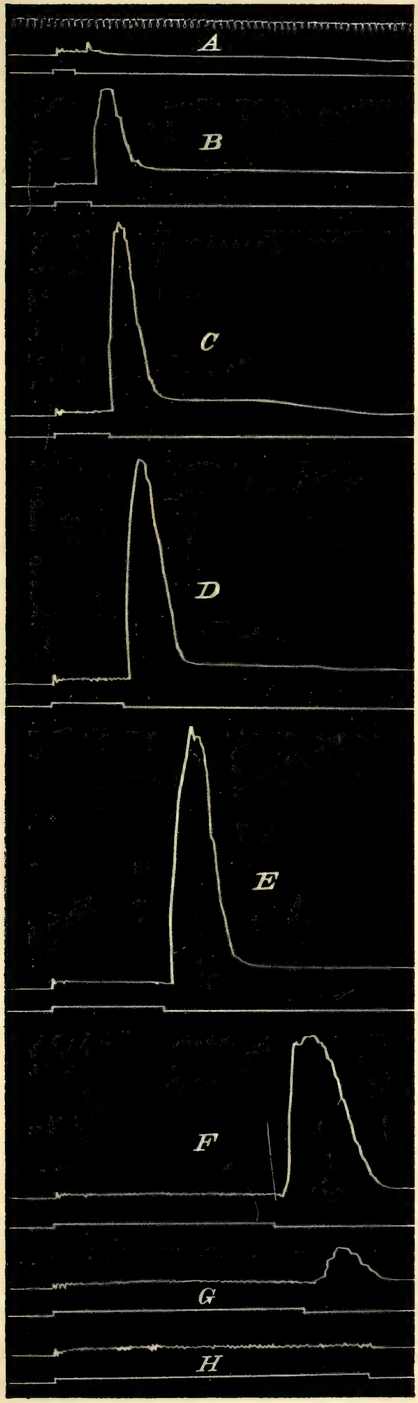

Fig. 4.

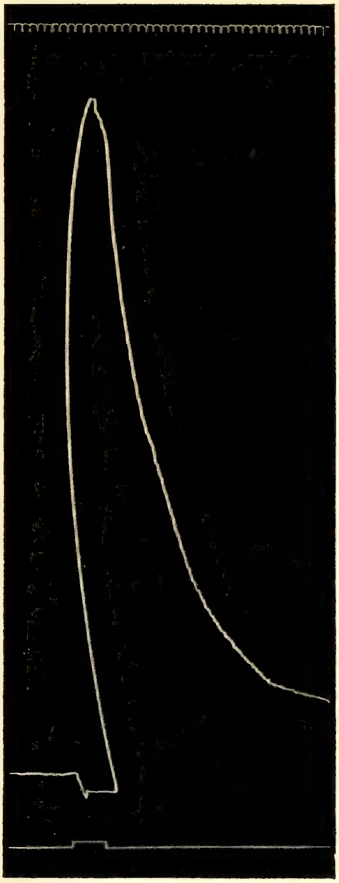

Fig. 5.

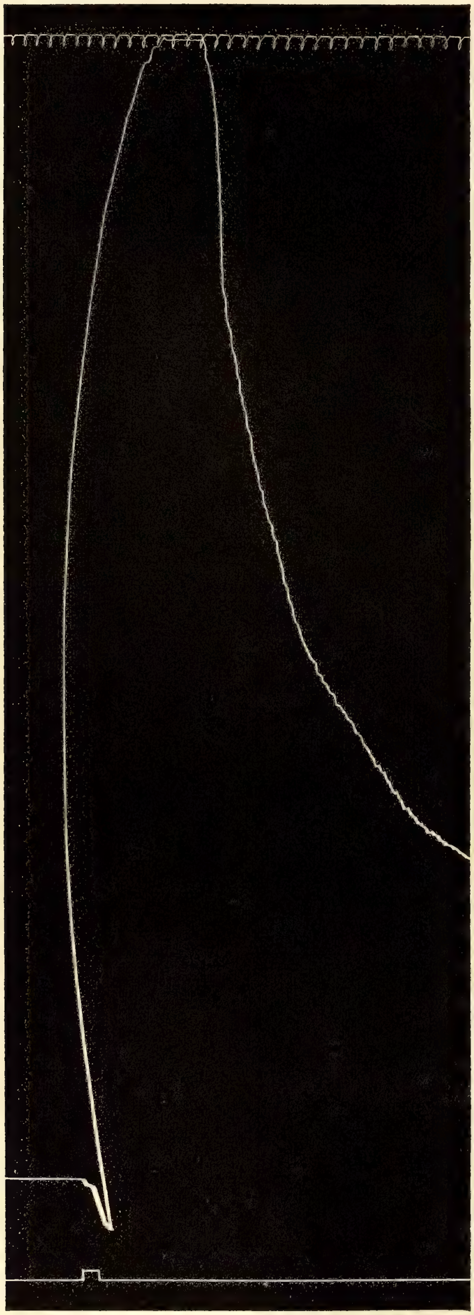

Fig. 6.

duration over which this holds good is wide, but it may be overstepped. An effective stimulus may, if much prolonged, pass unfollowed by contraction-phase (fig. 4, H). There seems for each intensity of stimulus a duration which best favours the occurrence and intensity of contraction-phase (fig. 4). The length of this period varies with the condition of the reflex preparation as well as with the intensity of the external stimulus. In fig. 4 the reflex preparation was of relatively low activity. The relation existing between the period of the stimulus and the development of contraction-phase recalls the observations of Bowditch and Warren* on the time-factor in the influence of precurrent stimuli from various sources on the amplitude of the knee-jerk; also the relation observed by Yerkes† between precurrent stimuli and a reflex elicitable from the frog.

The contraction of the contraction-phase varies from a brief spasm (fig. 3) to a prolonged tetanus lasting many seconds (fig. 8, A and B). The contraction may be of great amplitude and power. When intense it reaches its maximum with considerable speed (figs. 1 A, 6, 7, 8, A and B). Its decline may be rapid (figs. 3, 4), but more usually is quite gradual (figs. 1, 2, 7). Its decline is always, in my experience, more gradual than its ascent, and usually very greatly so (figs. 1, 2, 7). This is a point of difference between it and certain forms of strychnine reflex as exhibited by the same extensor muscles, and it constitutes a distinction of some importance for determining the relation of strychnine reflexes exhibited by these muscles to the normal after-phase of contraction which they also show.‡

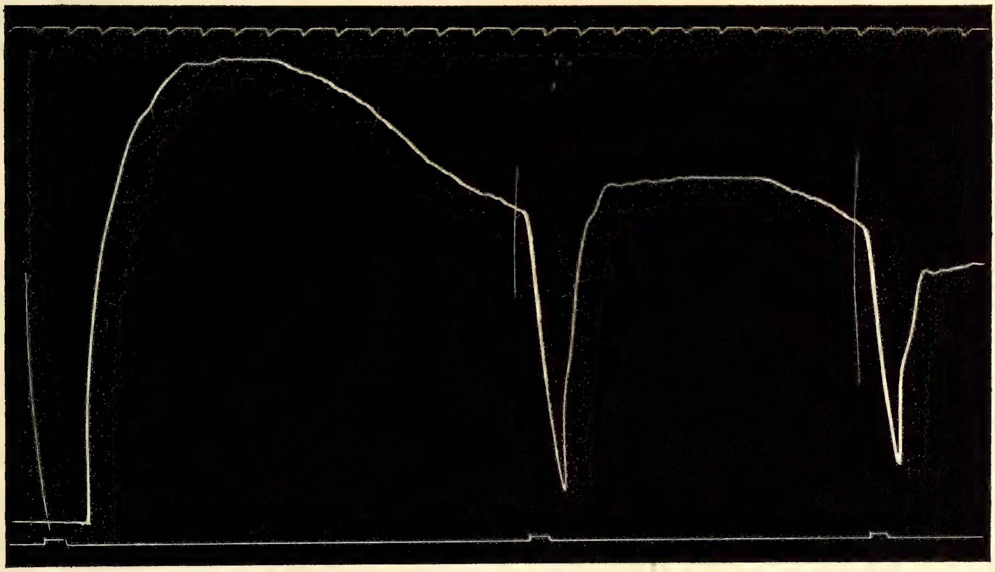

Fig. 7.

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* 'Journn. of Physiology,' vol. 11, p. 25, 1890.

† Pflüger's 'Archiv,' vol. 107, p. 207, 1905.

‡ Sherrington, 'Journ. of Physiol.,' vol. 36, p. 191, 1907.

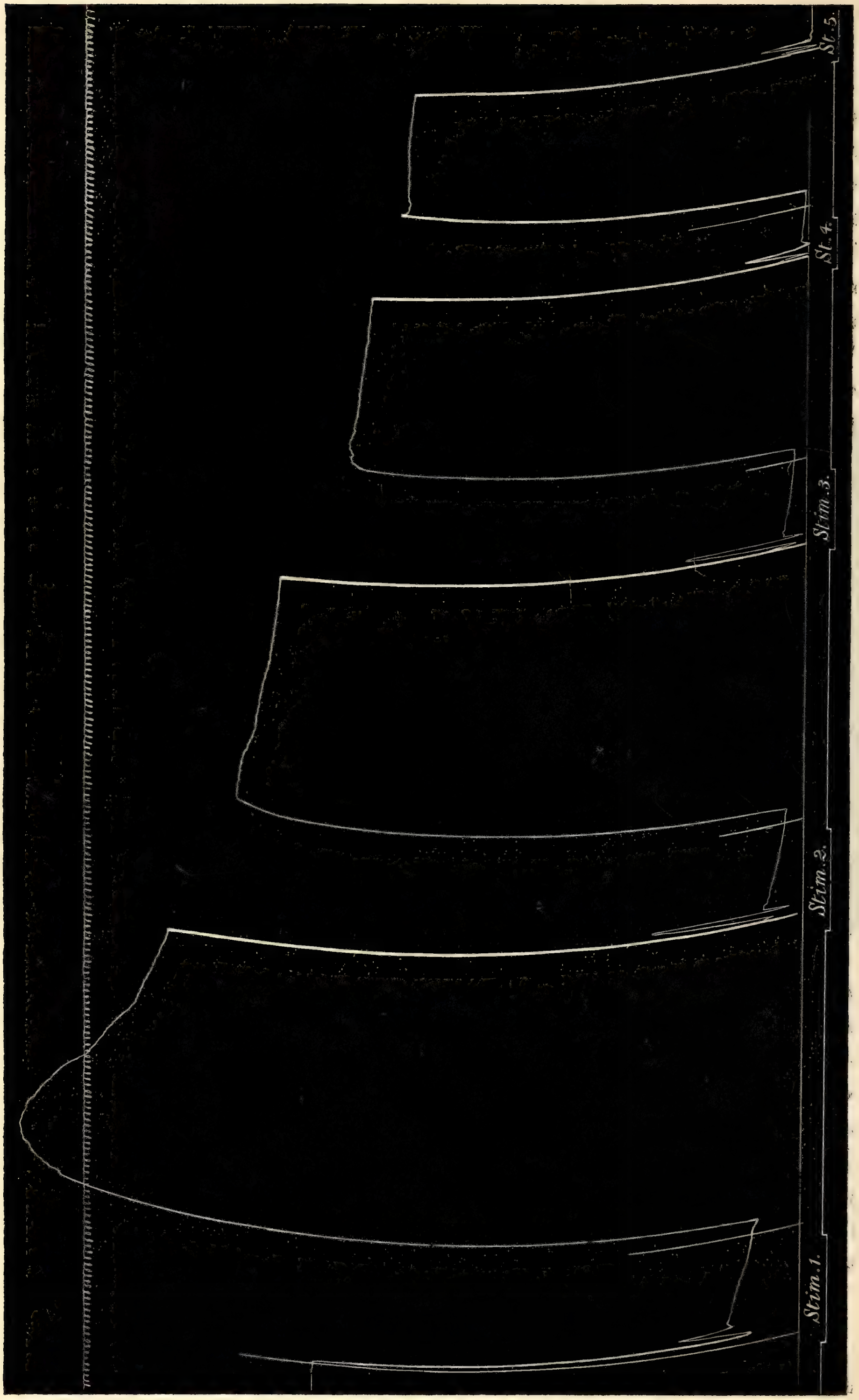

Fig. 8, A.

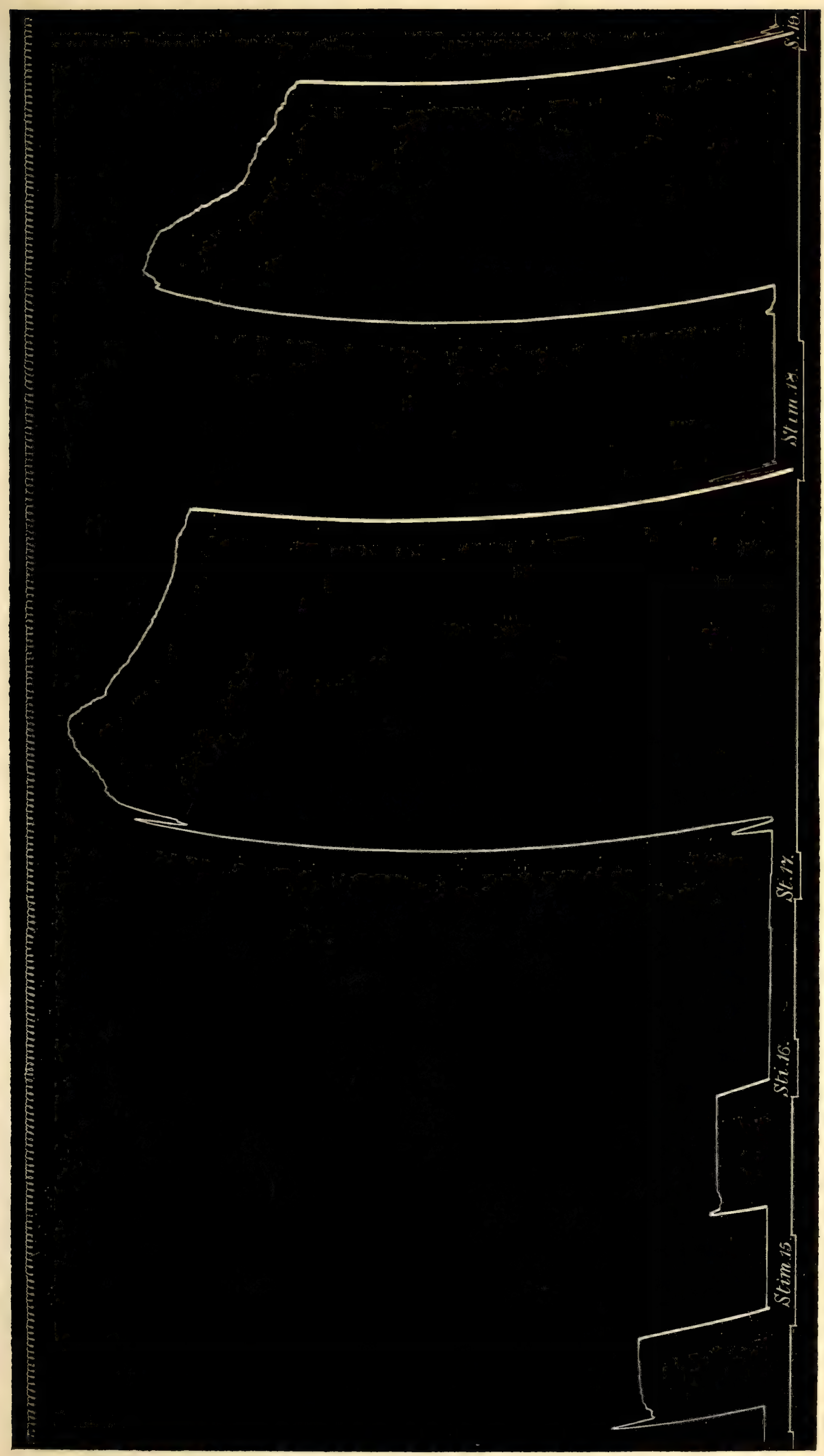

FIG. 8, B.

The contraction of the after-phase is extremely easily and rapidly cut short by a repetition of the stimulus upon which it followed. That stimulus inflicts on it with speed and certainty an inhibitory relaxation (figs. 7 and 8, A and B). Even the briefest repetition of the stimulus suffices. I have seen a repetition lasting only one-twentieth of a second cut it down. In fig. 7 is seen the effect of renewal of the stimulus for one-tenth of a second (fig. 7, third application of stimulus). The stimulus cuts it down by inhibitory relaxation, much as it cuts down a crossed extension reflex.*

On repetition of the original stimulus, the contraction of the contraction-phase is lessened or suppressed according to the intensity of the stimulus or the duration of its reapplication. On cessation of the reapplication the contraction-phase sets in again (figs. 7 and 8, A, B); subject only to the general conditions already mentioned as governing the contraction and to the condition that the reapplication has not been strongly repeated many times. The contraction-phase tires out more quickly than does the inhibition-phase. The contraction-phase that appears after a repetition of the stimulus does not seem to be simply a continuation or reappearance of the old contraction-phase that had been inhibited, for it often exceeds in intensity the intensity which the foregoing contraction-phase had at the moment when the inhibitory stimulus was renewed (fig. 7, second stimulation; and fig. 8, B). Nevertheless, the new contraction-phase has not so great intensity as the previous contraction-phase showed at outset (figs. 7 and 8, A). The fresh contraction-phase following each repetition of the inhibitory stimulus is on the whole somewhat less than the one preceding it (figs. 7 and 8, A, B), and the series of contraction-phases show progressive decline (figs. 7 and 8, A, B). Fig. 8, A, B, exhibits the first four and the last two of a series of 15 diphasic "flexion-reflexes" provoked in the extensor muscle (vasto-crureus) without pause between them. The external stimulus was not intense, and its intensity remained the same throughout this series, although the duration of its several applications was not exactly the same for each member of the series, since the short-circuiting key opened and shut by hand instead of automatically. The contraction-phase dwindled progressively in its repetitions, and on (fig. 8, B) withdrawal of the sixteenth stimulus no contraction-phase followed. The faradic stimulus was then (fig. 8, B) repeated with increased intensity, although with somewhat shorter duration. On cessation of this (seventeenth) stimulation, although little or no further lengthening of the already fairly-relaxed muscle had been obvious during the stimulation, a contraction-phase of considerable intensity at once followed (fig. 8, B). On repetition of the stimulus this was at once cut down. On withdrawing the stimulus contraction-phase again appeared almost as intense as on the next previous withdrawal (fig. 8, B). By repeating and withdrawing the stimulus a new series was obtained. This declined more rapidly than the former one, and when its contraction-phases had died out a further strengthening of the stimulus induced a new and still more rapidly-declining series. It is clear, therefore, that in the first series the contraction-phase had been exhausted only relatively to the intensity of the external stimulus used in that series. A stronger stimulus was still able to evoke more contraction-phase. I have shown a similar relation to exist between fatigue of reaction and intensity of stimulus in the scratch-reflex of the spinal dog.* On the whole, in my experience the contraction-phase is relatively little resistant to fatigue. It will often disappear for a given stimulus in three or four severe repetitions of that stimulus. It seems to wear out more rapidly than does the inhibition-phase of the same reflex. It wears out especially rapidly when the reflex excitability of the preparation is low, *e.g.*, under shock, general exhaustion, etc.

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* See fig. 3, in eighth Note of this series, 'Roy. Soc. Proc.,' B, vol. 76, p. 277.

It was said above that the contraction-phase is easily suppressed by chloroform narcosis. In the bulbospinal animal exhibiting extensor rigidity, a very considerable depth of chloroform or ether narcosis is required to abolish the rigidity. A depth of narcosis less than that required to abolish the rigidity of the muscles abolishes the contraction-phase of the "flexion-reflex" in them. When under deepening narcosis the stimulus for the "flexion-reflex" is repeated at suitable intervals, the contraction-phase following each inhibitory relaxation of the extensor muscle becomes less, and finally unobtainable before extinction of the decerebrate rigidity, and while the inhibitory relaxation of the muscle is still obtainable. Conversely, after chloroform narcosis has been pushed to the extreme of abolishing all reflex reaction, on lightening the narcosis the decerebrate rigidity reappears, and the reflex inhibitory relaxation of the extensors becomes demonstrable at a time when there is still no obtaining of the contraction-phase withdrawal of the inhibitory stimulus, occasioning no rebound contraction of the muscle.

Asphyxial conditions tend, as is well known, to augment some reflex reactions before finally paralysing them. The "flexion-reflex" is, as I have frequently seen in working with the spinal mammal, one of the reactions thus increased by impending asphyxia. It might, therefore, be thought that the appearance of the contraction-phase of the extensors in this reflex was attributable to a super-excitability, due to insufficient aeration of the blood from imperfect respiratory ventilation in the paralysed animal. This is not so; on the contrary, defective pulmonary ventilation and experimental conditions tending to asphyxia depress the contraction-phase, and, if allowed to continue, soon abolish it.

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* 'Journ. of Physiology,' vol. 34, p. 42, 1906.

The possible importance of "successive induction" as a coordinator of reflex actions seems obvious. If a reflex A not only temporarily inhibits a contraction B antagonistic to it, but also induces in the arc of B, as an immediately subsequent result, an excitation, we have a process qualified to link together simpler reflexes, so as to form from them reflex cycles of action such as characterise the reflex play of the limbs in locomotion. In a previous Note it was remarked that a difficulty in thus applying the experimental results then obtained lay "in the intensity and long duration" of the stimuli which have to be employed to produce the "successive induction."* "Such intensity and duration certainly do not occur in the course of the alternating reflexes"† as they occur naturally. The observations brought forward in the present communication remove that difficulty. Rebound contraction from "successive induction" is shown to be elicitable by stimuli of even momentary duration, *e.g.*, a single make or break shock; and still more readily by stimuli lasting even so brief a time as a twentieth of a second. The intensity of electric stimuli that evoke it need not be more than just perceptible to the tongue. Mechanical stimuli as well as electric also readily produce it.

The experiments afford, I think, convincing proof that in both fore limb and hind limb the ordinary "flexion-reflex," as expressed by the extensor muscles, is a diphasic reaction. The first phase is relaxation due to central inhibition, the second phase contraction due to central discharge. The first endures throughout the application of the external stimulus, the second immediately follows the withdrawal of that stimulus, and is due to a central rebound from the state of inhibition to a state of excitation. In the first phase of the reflex the extensor muscles abandon the maintenance of a posture, or the execution of a movement in which they were engaged; in the second phase they restore that posture to the limb, or reinstitute movement in the abandoned direction. The series of reflexes of fig. 8, A and B, is a series of "steps" executed by the limb under the sole action of a main extensor muscle of the knee (*vasto-crureus*). It has been shown‡ that in each reflex of such a series, the flexors§ contract during the application of the stimulus, that is, at and during time of inhibitory relaxation of the extensors.|| There-

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* Sherrington, 'Roy. Soc. Proc.,' B, vol. 77, p. 495.

† *Ibid.*

‡ Sherrington, 'Roy. Soc. Proc.,' vol. 52, 1893.

§ For list, see 'Roy. Soc. Proc.,' B, vol. 79, p. 341.

|| For list, *cf. ibid.*, p. 342.

fore, in a series of reflexes such as that of which fig. 8, A and B, shows the extensor muscle's reactions, the active movements of flexion and extension, alternating one with another, do not require alternation of two external stimuli, one evoking flexion, the other extension. Such observations show that one and the same stimulus, intermittently applied, suffices fully for the double phases of the reflex movement. Contraction-phase ensues in the muscle antagonistic to the flexor in each pause of application of the external stimulus. In other words, a neural discharge, due to successive induction, produces after each reflex contraction of the flexor a compensatory contraction of the extensor, bringing the limb back to extension and keeping it there until renewal of the external stimulus again inhibits the extensor, and contracts the flexor. In this case, unlike the "scratch-reflex," the rapidity of succession of the phases of the cyclic reflex is in the hands of the external stimulus. If the rate of intermission of that stimulus is slow, the alternation of the phases of the reflex is likewise slow; if the repetitions of the stimulus follow quickly, the cycle of the reflex proceeds quickly. That is to say, the rate of stepping of the limb depends on the rate of recurrence of the applications of a single uniform stimulus which excites, during each of its applications, one phase of the reflex act, and is followed by the other phase.

Addendum.—December 3, 1907.

(With Herbert E. Roaf, M.D. Toronto.)

(Received December 4, 1907.)

Mention was made in the foregoing* of the possibility that not only complete withdrawal of the inhibitory stimulus, but likewise mere lessening of its intensity, might induce from the inhibited centre fresh motor discharge and consequent contraction of the muscles previously kept relaxed by reflex inhibition. This proves to be actually the case. To obtain this effect under the experimental condition, the reduction of intensity of the external stimulus has had to be considerable, and the reduction has been abrupt. The method adopted has been that followed in studying the influence of the intensity of the external stimulus on the power of the stimulus to break through reflex fatigue.† The resistance of the primary circuit has been suddenly altered by introduction of further resistance of known amount. With the current and inductorium used, no rebound contraction was obtained under our conditions of experiment when the resistance was increased by less than 30 ohms. Fig. 9 shows the effect of abruptly reducing the current

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* *Cf.* also 'Journ. of Physiology,' vol. 36, p. 191.

† *Ibid.*, vol. 34, p. 42.

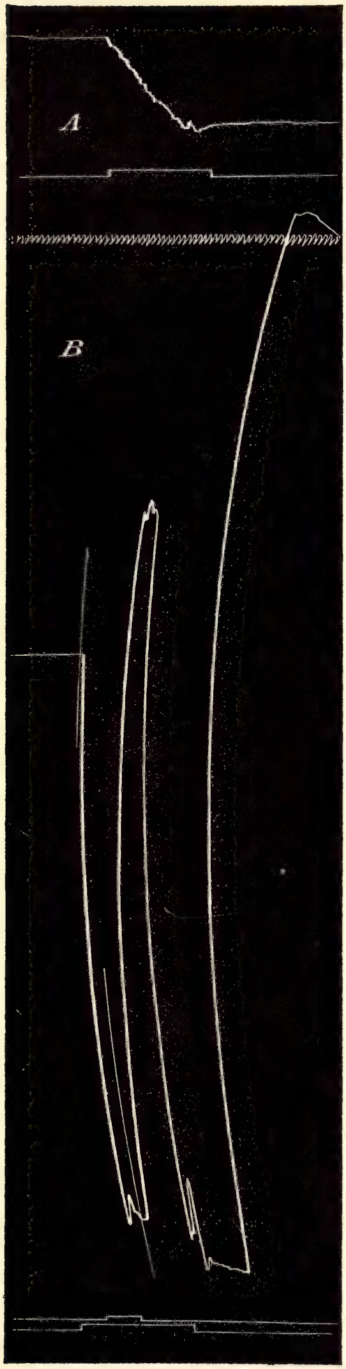

Fig. 9.

in the primary circuit by introducing 40 ohms additional resistance. The upper record (A) in the figure shows the reflex effect of faradisation of the afferent nerve with the secondary coil at 40 units of the Kronecker scale, and with the 40 additional ohms in the primary circuit. The effect is seen to be reflex inhibition, as is evidenced by a slight yet distinct relaxation of the vasto-crureus muscle. The stimulus was, therefore, above threshold value for producing reflex inhibition of the extensor arc. The lower figure opens with the effect of faradisation of the same nerve a minute later with the secondary coil unaltered in position (*i.e.*, 40 Kronecker units), but without the inclusion of the 40 additional ohms in the primary circuit. This stronger stimulus is seen to produce smart reflex relaxation of the extensor muscle. When that has reached its full amplitude and endured for something over a second, the extra 40 ohms are abruptly introduced (by un-short-circuiting them) into the primary circuit, and the faradic stimulus is thus reduced to the intensity it had in the upper trace of the figure, *i.e.*, to an intensity near, but distinctly above, threshold value under the experimental conditions of a minute before. The record shows that those conditions have now undergone a change, for now, instead of a weak inhibition, a strong extensor contraction sets in. This is the rebound contraction, and the change that has altered the conditions has been "successive induction" in the reflex preparation. The rebound contraction exceeds in intensity that obtaining prior to the inset of the original inhibition. The duration of the less intense faradisation, marked by the upper signal line, is continued for about a second, and then by short-circuiting the additional 40 ohms from the primary circuit, the more intense faradic stimulus is recontinued. The strong inhibitory relaxation of the extensor muscle at once returns. Finally, on discontinuing this, there occurs once more a rebound contraction of the extensor muscle, and this rebound is more intense still. Such observations show that the central rebound from inhibition to super-activity on the part of the reflex arc of the extensor takes place in the face of a weak inhibitory stimulus applied to the arc at the time of the rebound.

The result seems to have bearings of some theoretical importance. Of these one appears as follows. When reflex contraction has proceeded, even for a short while, under the application of a full exciting stimulus a weak excitatory stimulus, which prior to the strong reaction was able to excite the reflex contraction though weakly, loses some of its efficacy and becomes less able to provoke its reflex; in other words, tends to fall below the threshold value and may become subliminal.* Such a change in excitatory reflexes is ascribed to, or rather described under, fatigue effects. In the case of the inhibitory reflex just mentioned, an inhibitory stimulus which, prior to the action of a stronger inhibitory, was able to produce reflex inhibition, fails to do so when repeated after the stronger stimulus has been producing inhibition for a short time. If in the case of reflex excitation, causing contraction, the phenomenon is permitted to rank as fatigue, the similar result observed in reflex inhibition indicates that a similar wearing out or blunting of inhibitory effect occurs, and can, under like terminology, be called fatigue. There is thus emphasised one more of several significant likenesses between the converse processes of reflex excitation and reflex inhibition.

Another bearing of theoretical importance which the observation allows is the following. The flexion reflex is in reality the reflex stepping of the limb, and such stepping is a rhythmic alternating reflex. The observations in the present Note indicate that instead of having to look for two different alternately-acting stimuli wherewith to explain the two successive phases of flexion and extension characterising the reflex, one stimulus (namely, that exciting flexion) is all that may be needed. But that stimulus has to be intermittently applied, extension by rebound contraction occurring in the intermissions of its application. The additional observations in this addendum show that it is not necessary for the stimulus to be actually intermittent; it will suffice if it merely suffer periodic decrements of intensity—provided the decrements exceed a certain amount. In other words, in this reflex there is developed in the extensor neurones a slight refractory phase, slight because though effectively blocking a weak inhibitory stimulus, it fails before the insistence of a strong one. Thus analogy is at once revealed between this reflex and one superficially very different from it, namely, the "scratch-reflex." In the latter a feature of co-ordination is the occurrence of marked refractory phase. During the progress of the reflex the external stimulus which excites the contraction of the flexor muscles fails to excite them at rhythmically recurring periods owing to supervention of a refractory phase in the spinal centre. And the analogy is the closer since in the "scratch-reflex" the extensors of the limb as well as the flexors contract rhythmically,* and presumably their rhythmic contractions alternate with those of their antagonists. The recurrent refractory phase would therefore not only block the flexor excitatory stimulus, but at the same time block the extensor inhibitory stimulus. This is what it seems to be doing in the "step-reflex." The difference between the two reflexes becomes one of quantity and period rather than any essentially qualitative one.

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* Good instances are seen with the scratch-reflex, *cf.* 'Journ. of Physiology,' vol. 34, p. 1.

The facts indicate that the reflex movement of stepping, with its two opposite phases of flexion and extension, can be excited by one single form of stimulus intermittently or even merely unequally applied, that stimulus being the one which directly excites flexion. This suggests an explanation for the striking inequality with which flexion and extension respectively are represented in the receptive field, both deep and superficial, of the limb itself. The direct stimulation, electrical or mechanical (*i.e.*, ligation), of any afferent limb-nerve, whether cutaneous or deep, excites as its immediate result flexion of the limb itself, not extension.† Somewhat similarly, in the motor cortex (especially of the hind limb) the primary representation of flexion greatly preponderates over that of extension. The explanation of this curious inequality may lie in the circumstance that reflex flexion of the limb of itself induces as a sequence to itself extension, so that no local stimulus is, in so far, required for extension. The same argument may also apply to the analogous inequality of jaw opening and jaw closing, both as locally elicitable reflexes and in their primary representation in the "motor" cortex.* At root of the inequality with which movements of opposite direction, forming complemental pairs, are represented, both in the fields of local reflex action and in the "motor" *cortex cerebri*, may lie 'successive induction."

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* By appropriate isolation the following muscles in the cat are seen to exhibit the characteristic rhythmic contraction constituting the scratch-reflex—*tensor fasciæ femoris*, *psoas magnus*, *gluteus minimus*, front portion of *gluteus maximus*, *pectineus*, *sartorius*, *semitendinosus*, *tibialis anticus*, posterior portion of *biceps femoris*, *flexor longus digitorum*, *adductor parvus*, *vasto-crureus*, *semimembranosus* and anterior part of *biceps femoris*. Although most of these are flexor muscles, some, *e.g.*, the three last mentioned, are extensors. These three can be seen to contract simultaneously together in the rhythm of the reflex. For the rhythmic abduction which accompanies the rhythmic flexion of thigh in the reflex *gluteus minimus* seems chiefly responsible, since that rhythmic movement persists when all muscles of limb except *gl. min.* have been paralysed by severance of their nerves. This list probably does not exhaust the whole set of muscles exhibiting the rhythmic contraction of the reflex.—C. S. S.

† 'Integrative Action of the Nervous System,' p. 291, 1906; 'Roy. Soc. Proc.,' B, vol. 76, p. 293, and B, vol. 79, p. 337.

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* *Ibid.*, and 'Journ. of Physiology,' vol. 34, p. 315.

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Address of the President, Lord Rayleigh, O.M., D.C.L., at the Anniversary Meeting on November 30, 1907.

Since the last Anniversary the Society has sustained the loss of twenty-five Fellows and three Foreign Members.

The deceased Fellows are:—

Thomas Andrews,
Sir Benjamin Baker,
Sir Dietrich Brandis,
Sir William Henry Broadbent,
Dr. Alexander Buchan,
Lord Davey,
Dr. August Dupré,
Sir Joseph Fayrer,
Sir Michael Foster,
Sir William Tennant Gairdner,
Lord Goschen,
Sir James Hector,
Prof. Alexander Stewart Herschel,
Rev. John Kerr,
Sir Leopold McClintock,
Dr. Maxwell Tylden Masters,
Prof. Alfred Newton,
Cornelius O'Sullivan,
Sir William Henry Perkin,
Dr. William Henry Ransom,
Sir Edward James Reed,
Dr. Edward John Routh,
Henry Chamberlaine Russell,
Prof. Charles Stewart,
Robert Warington.

The Foreign Members are:—

Marcellin Berthelot, Dmitri Ivanovitch Mendeleeff, Henri Moissan.

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* *Ibid.*, and 'Journ. of Physiology,' vol. 34, p. 315.

From the length of this list it will be seen that death has been unusually busy during the year. It includes many names of great distinction, of whom I can refer only to a few.

Sir B. Baker was a Councillor at the time of his death, and was well known among us as a frequent attendant at our meetings and as combining scientific interests with the highest degree of successful practice in his profession. The recent catastrophe in America will, perhaps, even enhance his reputation as the designer of the Forth Bridge.

In Sir Michael Foster we have lost one, to whom probably more than to anyone else the present position of our Society is due. For 22 years he held the office of Secretary, during about half of which time I was his colleague. It would not be too much to say that the interests of the Society and a desire to extend its usefulness were never out of his mind. It was inevitable that his pronounced views and his energy in giving effect to them should occasionally arouse opposition, but he was impelled always by public spirit, sometimes to the detriment of his own private interests. His work for Cambridge, for the Government in commissions of inquiry, as well as for the Society, constitute a lasting claim upon the gratitude of our generation.

The name of Kerr will go down to posterity as the discoverer of two remarkable phenomena in Electro-Optics. His success is a good example of what may be accomplished under no small difficulties by courage and perseverance.

The claims of Sir W. Perkin as the pioneer in the aniline industry and as a distinguished worker in scientific chemistry have recently been celebrated, and are recognised all over the world. It is satisfactory to reflect that, unlike many inventors, he met with full appreciation during his lifetime.

Dr. Routh's name is one that I cannot allow to pass without a word. I was indebted to him for mathematical instruction and stimulus at Cambridge, and I can still vividly recall the amazement with which, as a freshman, I observed the extent and precision of his knowledge, and of the rapidity with which he could deal with any problem presented to him. His book on Dynamics is well known. In its earlier editions it illustrated, perhaps, rather the vices than the virtues of the Cambridge School, but it developed later into a work of first-rate importance. I have always been under the impression that Routh's scientific merits were underrated. It was erroneously assumed that so much devotion to tuition could leave scope for little else.

On the foreign list we have to lament three chemists of high distinction, Berthelot and Mendeleeff, though still active, had attained old age; but in Moissan we lose one from whom much more might have been expected. All three have been recipients of our medals.

An important feature in the work of the Royal Society consists of various enquiries, undertaken for different Departments of Government, in regard to diseases which affect the tropical portions of our foreign possessions and dependencies. Among these diseases the attention of the civilised world has been for some years directed to the malady known as Sleeping Sickness. The first concerted action for the study and combating of this apalling scourge arose out of a representation made by the Royal Society to the Foreign Office in the spring of 1902, in consequence of which, at the request of the Treasury, the Society's Malaria Committee organised and despatched a small scientific commission to Uganda. In the course of a short time the source of the disease was traced by this Commission to the presence of a trypanosome in the blood and cerebro-spinal fluid of the victims, and the further discovery was also made by the same Commission that the trypanosomes are carried by a species of biting tsetse-fly. These important revelations were followed up by detailed studies of the character and distribution both of the disease and of the fly. Besides sending out a succession of observers to prosecute the investigations of its Commission at Entebbe, the Royal Society urged upon the Colonial Office the necessity of organising, and under an increased medical staff, a more comprehensive enquiry into the local conditions under which the disease is propagated. This recommendation was carried out and some valuable information on the subject has been obtained. Meanwhile, though various drugs had been tried with at best only temporary success, no lasting remedy had been found for the malady, which has continued to be fatal and to spread steadily over Central and East Africa. The various European Governments which have possessions in those regions have at last determined to make a united effort to cope with Sleeping Sickness through the instrumentality of an International Conference having a separate bureau in each country concerned and a central bureau in London. The object of this co-operation will be to collect information bearing on the disease, to devise and carry out such scientific researches as may seem to be necessary and to concert measures for dealing with the disease and the populations affected or likely to be affected by it. The Royal Society, having led the way in this subject, has been invited to give the proposed combined international action its support. The Society welcomes the proposal and will be prepared to render every assistance in its power. In the meantime our Tropical Diseases Committee is continuously and actively engaged in the endeavour to discover a drug that may prove effective in the treatment of the disease. Their investigations have been directed to the study of trypanosomiasis in rats and the latest results obtained are such as to encourage the hope that at least in this direction their labours have been successful.

During the present year three parts of the Reports of the Society's Mediterranean Fever Commission have been published, embodying the final observations and conclusions in this important enquiry, which was undertaken at the joint request of the Admiralty, War Office, and Colonial Office. It is not often that a difficult investigation of this kind can be brought to a successful conclusion in so short a time as two years and a-half, and the various members of the Commission are deserving of the warmest commendation for the skill, zeal, and promptitude with which they have solved the problem submitted to them. They have shown how the scourge of fever, which has been so long rife in Malta, and has so seriously reduced the strength of our garrison there, may be eventually banished from the island. Already their recommendations, so far as they have been followed, have reduced the amount of fever to trifling proportions. It now remains for the authorities to adopt the further precautions pointed out to them, which will probably banish the disease altogether.

I have continued to preside at the Meetings of the Executive Committee of the National Physical Laboratory.

The work of the Laboratory has grown greatly during the year. The addition to the Engineering Building and the new building for Metallurgical Chemistry are completed and are now occupied, while the building for Metrology is very nearly finished.

A new 100-ton testing machine, one of Messrs. Buckton's latest patterns, has been installed, and the increased accommodation in the Engineering Laboratory enables the work there to proceed more easily and rapidly.

Great progress has been made in the equipment of the Electro-technical Laboratory, and research and test-work can now go on there in a rapid and systematic manner.

The question of the Commercial Testing undertaken by the Laboratory has been the subject of investigation by a Treasury Committee, before which I was summoned to give evidence. It is understood that the report of this Committee may be expected shortly.

Progress has been made with the buildings at Eskdale Muir, some of which are now ready for occupation. It was hoped that the work might have begun this summer, and the Treasury have provided a sum of £750 for the expenses during three-quarters of the current financial year. Owing to the bad weather in the early summer this anticipation has not been realised, but a start will be made very shortly. The buildings are admirably adapted for their purpose, and will render possible the study of terrestrial magnetism under the undisturbed conditions which used to exist at Kew.

A list of the more important researches is published in the Report of the

Laboratory. Among these may be mentioned those by Dr. Harker on the Kew Scale of Temperature and its relation to the International Hydrogen Scale; Mr. Paterson's paper, read before the Institution of Electrical Engineers—"Investigations of Flame Standards and the Present Performance of High-voltage Lamps"; and the eighth report of the Alloys Research Committee, by Dr. Carpenter and Mr. Edwards, on the Properties of Alloys of Aluminium and Copper. Professor Ayrton, Mr. Mather, and Mr. Smith have finished their work on the Ampere Balance, and the paper is now being published by the Royal Society in the 'Philosophical Transactions,' while papers on the Silver Voltameter and the Weston Cell are also in the press.

Dr. Stanton and Mr. Bairstow have completed a further research on the measurement of wind pressure, and are well advanced with the investigation into methods of impact-testing.

Other researches in progress are those on the measurement of small inductances and capacities, with a view to the standardisation of the wave-lengths used in wireless telegraphy, on alloys of copper, aluminium, and manganese, for the Alloys Research Committee, and on the properties of eutectics.

The completion of the work on the electrical units will be satisfactory to those who have been interested in this question. At the time of my own researches about twenty-five years ago, the ohm and the ampere were uncertain to 2 or 3 per cent., and I then scarcely hoped to get nearer than one part in a thousand. The recent work carried on at Bushey would seem to indicate that an accuracy of one part in ten thousand may have been attained. The possibility of such a refinement depends largely upon the use in the instruments of coils composed of a single layer of wire, the position of every turn of which is open to exact determination. The importance of this feature was insisted upon by the late Professor Jones.

Accuracy of measurement appeals less to the lay and scientific public than discoveries promising to open up new fields; but though its importance at any particular stage may be overrated, it promotes a much needed consolidation and security in the scientific edifice. A remarkable example of enhanced accuracy is afforded by modern measurements of luminous wave-lengths, for which we are mainly indebted to our Copley medallist. Not only did he introduce the vacuum tube charged with mercury or cadmium as the best source of homogeneous light, but by a most able use of an ingenious method he determined, with the highest precision, the values of the cadmium red, green, and blue wave-lengths in terms of one another, and of the metre. His work has been skilfully followed up by Fabry and Perot, and numerous wave-lengths are now known with a relative accuracy of onemillionth part. When we reflect upon the almost ultra microscopic magnitude of a wave-length of light, the possibility of such an achievement may well excite our astonishment.

For the advancement of science the main requirement is, of course, original work of a high standard, adequately explained and published. But this is not enough. The advances so made must be secured, and this can hardly be, unless they are appreciated by the scientific public. In some branches of pure Mathematics it is said that readers are scarcer than writers. At any rate the history of science shows that important original work is liable to be overlooked and is, perhaps the more liable, the higher the degree of originality. The names of T. Young, Mayer, Carnot, Waterston, and B. Stewart, will suggest themselves to the physicist; and in other branches, doubtless, similar lists might be made of workers whose labours remained neglected for a shorter or a longer time. In looking into the more recent progress of Geometrical Optics, I have been astonished to find how little correlation there has been between the more important writings. That Coddington should have remained unknown in Germany and von Seidel in England need not greatly surprise us; but in this subject it would appear that a man cannot succeed in making even his own countrymen attend to him. Coddington seems to have heard nothing of Cotes and Smith, and Hamilton nothing of Airy and Coddington.

It is true that no two writers on theoretical subjects could differ more in taste and style than do Hamilton and Coddington. The latter addressed himself to special problems, the solution of which seemed to have practical importance. Among his achievements was the rule relating to the curvature of images, generally known as Petzval's, although Petzval's work was of much later date. Hamilton, on the other hand, allowed his love of generality and of analytical developments to run away with him. In his Memoir on Systems of Rays, with its elaborate and rambling supplements, there is little to interest the practical optician, though the mark of genius is throughout apparent. It was only in two or three pages of a later paper that he applied his powerful methods to the real problem of Optics. As Finsterwalder has remarked, his "six radical constants of aberration," expressing the general properties of a symmetrical instrument, are at once an anticipation and a generalisation of von Seidel's theorems. But the published work is the barest possible summary. If Hamilton had been endowed with any instinct for Optics proper, he could nave developed these results into a treatise of first-class importance. In more recent times Hamilton's footsteps have been followed by Maxwell as well as by Thiesen and Bruns, of whom the two latter do not seem to have realised that Hamilton (or even Maxwell) had concerned himself with the subject at all. The natural development of Hamilton's ideas will be found in an able memoir by Schwarzschild (1905).

I have spoken of English work that lay neglected, but a scarcely less notable instance is the splendid discovery of the microscopic limit by Fraunhofer, a man who combined in the highest degree practical skill with scientific insight. Thanks to the researches of Abbe and Helmholtz, it is now well known that there is a world that lies for ever hidden from our vision, however optically aided; but neither of these eminent men realised that the discovery had been anticipated by Fraunhofer. Some, perhaps, may doubt whether Fraunhofer's argument, founded upon the disappearance of spectra from gratings of extreme fineness, is of adequate cogency. To this I may reply that I was myself convinced by it in 1870, before either Abbe or Helmholtz had written a word upon the subject.

Enough has probably been said to illustrate my contention that much loss has ensued from ignorance and neglect of work already done. But is there any remedy? I think there ought to be. In all the principal countries of the world we have now a body of men professionally connected with science in its various departments. No doubt the attention of many of these is so engrossed by teaching that it would be hard to expect much more from them, though we must remember that teaching itself takes on a new life when touched with the spirit of original enquiry. But in the older Universities, at any rate, the advancement of science is one of the first duties of Professors. Actual additions to knowledge occupy here the first place. But there must be many who, from advancing years or for other reasons, find themselves unable to do much more work of this kind. It is these I would exhort that they may fulfil their function in another way. If each man would mark out for himself a field—it need not be more than a small one—and make it his business to be thoroughly conversant with all things new and old that fall within it, the danger of which I have spoken would be largely obviated. A short paper, a letter to a scientific newspaper, or even conversation with friends and pupils, would rescue from oblivion writings that had been temporarily overlooked, thereby advancing knowledge generally and sometimes saving from discouragement an unknown worker capable of further achievements. Another service such experts might render would be to furnish advice to younger men desirous of pursuing their special subject.

The readers of whom I have been speaking are experts capable of advancing science themselves and of helping others to do so. But there is another class of possible readers of scientific books on behalf of whom I wish to make an appeal. We who are dependent upon sight in almost everything that we do must specially sympathise with those unfortunates who are deprived of this most precious gift. A movement is on foot, and has already received valuable support, to promote the publication of standard scientific works in embossed type suitable for the use of the blind. Such publication is costly and can hardly be undertaken upon an adequate scale without external aid. My friend, Mr. H. M. Taylor, a Fellow of this Society, tells me that in the course of the last 12 months he has written out the whole of Mr. C. Smith's Elementary Algebra in Braille type, has afterwards read the copy with his fingers and again, later, read the whole in proof. There can be no doubt that books in embossed type on such subjects as Mechanics, Physics, Astronomy, Geology, not to mention the various Biological Sciences, would be an immense boon to many blind readers. I commend the proposal heartily to your notice.

Another remedy for the confusion into which scientific literature is liable to fall may lie in the direction of restricting the amount of unessential detail that is sometimes prevalent in the publication of scientific results. In comparing the outputs of the present time, and of, say, 30 years ago, the most striking feature that appears is doubtless the increase of bulk, in recent years coming especially from young workers stimulated by the healthy encouragement of direct research as a part of scientific education. But I think it may also be observed, and not alone in the case of such early dissertations, that there is, on the whole, less care taken for the concise presentation of results, and that the main principles are often submerged under a flood of experimental detail. When the author himself has not taken the trouble to digest his material or to prepare it properly for the press, the reader may be tempted to judge of the care taken in the work from the pains taken in its presentation. The tendency in some subjects to submit for immediate publication the undigested contents of note-books is one that we hear much of at the present time. It is a matter that is difficult for publishing bodies to deal with, except by simple refusal of imperfectly prepared material, with its danger of giving offence to authors of recognised standing, but it seems not unlikely that at present public scientific opinion would endorse such a course of action. A related difficulty and one that contributes to this trouble, is the tendency, noticeable in some public scientific organisations, to imagine that their activity is estimated by the number of pages of printed matter they can produce in the year. Probably no consideration is further removed than this from the minds of the educated public, whose judgment is alone worth considering.

MEDALLISTS, 1907.

COPLEY MEDAL.

The Copley Medal is awarded to Professor Albert Abraham Michelson, For. Mem. R.S., on the ground of his experimental investigations in Optics.

In 1879, Michelson brought out a determination of the velocity of light by an improved method, based on Foucault's, which gave 299,980 kilometres per second. Three years later, by means of a modification of the method, capable of even greater precision, he found for this constant, of fundamental importance for electric as well as optical science, the value of 299,853 kilometres.

Michelson has been a pioneer in the construction of interferometers, which are now indispensable in Optics and Metrology. With his new instrument, at Paris, he determined the absolute wave-lengths of the red, green, and blue lines of cadmium by counting the number of fringes (twice the number of wave-lengths) corresponding to the length of the standard metre of the Bureau International des Poids et Mesures. He found the metre to be 1,553,164 times the wave-length of the red line of cadmium, a result which is almost in exact agreement with the redetermination last year by Perot and Fabry. Michelson thus proved the feasibility of an absolute standard of length, in wave-lengths, of such accuracy, that if the standard metre were lost or destroyed, it could be replaced by duplicates which could not be distinguished from the original.

He had the greatest share in the elaboration of precise experiments on the relative motion of ether and matter. He repeated in an improved form Fresnel's experiment of the speed of light in moving media, using water and sulphide of carbon. He found that the fraction of the velocity of the water by which the velocity of light is increased is 0·434, with a possible error of ± 0·02. The fact that the speed is less in water than in air shows experimentally that the corpuscular theory is erroneous; but his results, moreover, established the correctness of Fresnel's formula for the effect, the theory of which has since become well understood.

In conjunction with E. W. Morley, he devised and carried out a very remarkable method by which, on the assumption of ether at rest, an effect depending on quantities of the order $(v/V)^2$ would appear to be appreciable. No displacement of the fringes was found. Of this result the simplest explanation would be that the ether near the earth partakes fully in its orbital motion; but modern electric and optical science appears to demand a quiescent ether, and the existence of this and similar null results is fundamental for its theory.

He has shown the possible application of the Interferometer method to Astronomy, by himself measuring the diameters of the four satellites of Jupiter, which are only about one second of arc. He suggests the further application of the instrument to such of the fixed stars as may not subtend less than one-hundredth of a second of arc.

In 1898, Michelson constructed a spectroscope which enables us to make use of the great resolving powers of the very high orders of spectra which are absent in the use of the ordinary grating, and with the added advantage of having most of the light in one spectrum. The echelon consists of a pile of glass plates of precisely equal thickness, which overlap by an equal amount; with it spectral lines which appear single with the most powerful gratings can be resolved into components. This instrument has been especially useful for the direct observation of the important, because definite, influence of magnetism on light, discovered by Zeeman. With 30 plates, and using the 25,000th spectrum, the echelon has a resolving power of 750,000, while the most powerful gratings do not exceed 100,000.

In connection with the analysis of radiations, he has constructed and used various machines for the analysis of periodic motions. For example, in conjunction with Stratton, he perfected a remarkable machine which is based on the equilibrium of a rigid body under the action of springs.

Professor Michelson has also investigated by his Interferometer the important subject, both theoretically and practically, of the breadth and the structure of spectral lines, including the effect of a magnetic field, and in various other ways his genius has opened up new ground in experimental Optics.

Royal Medals.

One of the Royal Medals has been awarded, with the approval of His Majesty, to Dr. Ernest William Hobson, F.R.S.

During the last 20 years Dr. E. W. Hobson has been distinguished for the fundamental character of his contributions to Mathematics and Mathematical Physics. His earlier published work, from 1888 onwards, deals largely with the so-called Harmonic Analysis, which embraces many topics having for their common aim the solution of the Potential Equation in forms suitable for application to the problems of Physics. The exhaustive examination of the general types of Harmonic Functions contained in his paper in the ‘Phil. Trans.,’ 1896, has been found to be of high utility for this application. He was led by these researches, and particularly by the difficulty of describing in general terms the characteristics of a function capable of being represented by Fourier's series, to take part in the revision of the logical basis of

Differential and Integral Calculus which is now in progress; his Presidential Address to the London Mathematical Society, in 1902, on the questions here arising, aroused general interest among mathematicians; and he has recently (1907) published an extensive volume, dealing with the whole matter and its applications to the theory of Fourier's series, which is of great importance for the history and development of Mathematics.

His Majesty has also approved the award of a Royal Medal to Dr. Ramsay H. Traquair, F.R.S.

Dr. Traquair is honoured on the ground of his long continued researches on the fossil fishes of Palæozoic strata, which have culminated, within the past 10 years, in his discovery of new groups of Silurian and Devonian fishes, and in his complete exposition of the structure of *Drepanaspis*, *Phlyctenaspis*, and other remarkable forms.

For nearly forty years Dr. Traquair has been busy with the description of fossil fishes, mostly from the Palæozoic rocks of Scotland, and he is deservedly held to be one of the most eminent palæontologists of the day. He has been highly successful in the interpretation of the often very obscure and fragmentary remains which he has had to elucidate, and his restorations of fishes have won such credit as to appear in all modern text-books of Palæontology. It may be said that his work, notwithstanding the great difficulties of the subject, has well stood the test of time.

Dr. Traquair has done much to advance our knowledge of the osteology of fishes generally. His earliest memoirs on the asymmetrical skull of flat-fishes and on the skull of *Polypterus* remain models of exactness. His acquaintance with osteology enabled him to show how former superficial examination of the Palæozoic fishes had led to wrong interpretations. He demonstrated that *Chirolepis* was not an Acanthodian, as previously supposed, but a true Palæoniscid. In 1877 he satisfactorily defined the Palæoniscidæ and their genera for the first time, and conclusively proved them to be more nearly related to the Sturgeons than to any of the other modern Ganoids with which they had been associated. He thus made an entirely new departure in the interpretation of extinct fishes, replacing an artificial classification by one based on phylogenetic relationship. His later memoir on the Platysomidæ was equally fundamental and of the same nature.

All subsequent discoveries, many made by Traquair himself, have confirmed these conclusions, which are now universally accepted.

In 1878, Dr. Traquair demonstrated the Dipneustan nature of the Devonian *Dipterus*, and somewhat later he began the detailed study of the Devonian fishes. His latest researches on the Upper Silurian fishes of Scotland are equally important, and provide a mass of new knowledge for which we are indebted to his exceptional skill and judgment in unravelling the mysteries of early Vertebrate life.

DAVY MEDAL.

The Davy Medal is awarded to Professor Edward Williams Morley.

Professor Edward W. Morley is well known both to chemists and to physicists for his work in the application of optical interferences and other physical phenomena to increase the accuracy of measurement. Numerous valuable papers have appeared, either in collaboration with Professor Michelson and others, or in his own name, on such subjects. Special reference may be made to his experiments, in conjunction with Professor Michelson, on the fundamental question of the absence of effect of translatory motion of material bodies on luminous phenomena.

His claim to the Davy Medal rests on grounds closely related to these researches; for he has combined thorough mastery of accurate measurement with an intimate knowledge of modern chemistry, and has utilised them in his attempt to solve one of the most difficult and fundamental problems of chemical science. The special problem to which he has consecrated many years of his life is the determination of the relative atomic weights of hydrogen and oxygen; it has been attacked by him with rare insight and skill, and with indomitable perseverance, and he seems to have settled it for many years to come, if not permanently. All the recent work devoted to this problem, and there has been much, has tended to establish more firmly the ratio arrived at by Professor Morley.

His determinations of the absolute weights of a litre of hydrogen and of oxygen, and his investigations of the amounts of moisture retained by gases dried by various desiccating agents, are of the very greatest importance for scientific progress.

SYLVESTER MEDAL.

Professor Wilhelm Wirtinger, of Vienna, is the recipient of the Sylvester Medal.

He is distinguished for the importance and wide scope of his contributions to the general Theory of Functions. Our knowledge of the general properties and characteristics of functions of any number of independent variables, and our ideas for the further investigation of such functions are, for the most part, at present bound up with the Theory of Multiply-periodic Functions, and this Theory is of as great importance for general Solid Geometry as the ideas of Abel have proved to be for the Theory of Plane Curves. Professor Wirtinger has applied himself for many years to the study of the general problems here involved. A general summary of his researches is given by him in the Abel Centenary volume (xxvi, 1902) of the 'Acta Mathematica.' Two of his papers may be particularly referred to, both of 1895. One of these deals with the reduction of the Theory and General Multiply-periodic Functions to the Theory of Algebraic Functions, with a view to their expression by Theta functions; this was one of the life problems of Weierstrass, who did not, however, during his lifetime, publish anything more than several brief indications of a method of solution. Professor Wirtinger's memoir obtains a solution, and is, moreover, characterised throughout by most stimulating depth and grasp of general principles. This paper was followed by two others, one continuing the matter in detail, the other making an application of its principles to the general Theory of Automorphic Functions. Another extensive paper, which obtained the Beneke Prize of the Royal Society of Göttingen, deals with the general Theory of Theta Functions. In it he obtained results of far-reaching importance, for Geometry as well as for the Theory of Functions, the full development of which will require many years of work.

HUGHES MEDAL.

The Hughes Medal is awarded to Principal Ernest Howard Griffiths.

Principal Griffiths has conferred great benefit on physical science by his series of measurements of fundamental constants, mainly in the domain of thermal and electric energy. At a time when the equivalent of the thermal unit in mechanical energy stood urgently in need of revision, he devoted himself to the problem with all the refinements and patient manipulation that could be devised, the result being a value for Joule's equivalent which at once acquired authority in the light of the evidence produced, and largely confirmed the corrections already advanced by Rowland and others. A main cause of discrepancy had been found to be the variation of the thermal capacity of water with the temperature; and by an investigation in which this variation was determined, Griffiths elucidated and correlated fundamentally the work of previous observers, from Joule onward. Of special importance also, in the domain of chemical physics, was an investigation of the depression of the freezing point of water by very dilute admixture of dissolved substances, wherein he verified, with all the refinement of absolute physical determinations, that the change of freezing point ran exactly parallel to the electric conductivity when the dilution of the electrolysable salt was comparable to that of gases, being twice as much per molecule as the standard value of the depression for non-electrolytes.

BUCHANAN MEDAL.

The Buchanan Medal is awarded to Mr. William Henry Power, C.B., F.R.S. Mr. Power's services to Hygienic Science and Practice have extended over a period of more than thirty years, and have been of the most distinguished kind. He has himself personally conducted successful enquiries into the causes of the spread of various diseases, and has obtained results which have proved of the greatest benefit to mankind. Moreover, in his long connection with the medical department of the Local Government Board, he has planned and directed numerous general and local investigations whereby our knowledge of disease, and of the methods of coping with it, have been greatly increased. The medical reports issued by the Local Government Board, which are universally regarded as among the most important contributions of our time to this subject, have for many years past been either written by him or owe much to his editorial criticism and supervision. It is not too much to say that no living man in this country has advanced the cause of scientific hygiene more than Mr. Power, or is more worthy of the distinction of the Buchanan Medal.

On the Inheritance of Eye-colour in Man.

By C. C. Hurst.

(Communicated by W. Bateson, F.R.S. Received May 7,—Read November 14, 1907.)

The following notes on the inheritance of eye-colour in man are based on material examined by the writer in the village of Burbage, Leicestershire. During the past three years, 139 pairs of parents and 683 of their offspring have been examined, with the following results:—

Eye-colour in man depends almost wholly on the colour of the iris. The colour of the iris varies considerably in different families and often in different individuals of the same family. This variability is due partly to the presence or absence of different pigments on the anterior and posterior surfaces of the iris, and partly, in all probability, to actual differences in the structure of the iris.

In the Report of the Anthropometric Committee of the British Association for 1880, the following statement concerning the nature of eye-colour in man is given by Mr. Charles Roberts (pp. 134 to 136):—

"The iris, on which the colour of the eye depends, is a thin membranous structure, composed of unstriped muscular fibres, nerves, and blood-vessels, held together by a delicate network of fibrous tissue. On the inner surface of this membrane there is a layer of dark purple pigment called the *uvea* . . . and in brown eyes there is an additional layer of yellow (and, perhaps, brown-red) pigment on its outer surface also, and in some instances there is a deposit of pigment amongst the fibrous structures. In the albino where the pigment is entirely absent from both surfaces of the iris, the bright red blood is seen through the semi-transparent fibrous tissues of a *pink* colour; and in blue eyes, where the outer layer of pigment is wanting, the various shades are due to the dark inner layer of pigment—the *uvea*—showing through fibrous structures of different densities or degrees of opacity.

"The eyes of new-born infants . . . are dark blue, in consequence of the greater delicacy and transparency of the fibrous portion of the iris; and as these tissues become thickened by use, and by advancing age, the lighter shades of blue and, finally, grey are produced, the grey, indeed, being chiefly due to the colour of the fibrous tissues themselves. In grey eyes, moreover, we see the first appearance of the superficial layer of yellow pigment in the form of isolated patches situated around the margin of the pupil, or in rays running across the iris.

"In the various shades of green eyes the yellow pigment is more uniformly diffused over the surface of the iris, and the green colour is due to the blending of the superficial yellow pigment with the blue and grey of the deeper structures. In the hazel and brown eyes the *uvea* and the fibrous tissues are hidden by increasing deposits of yellow and brown pigment on the anterior surface of the iris, and when this is very dense, black eyes are the result."

The above statement agrees well with my own observations, though I would add that the presence of the superficial layer of yellow pigment, when only slightly developed, is to be seen in blue eyes as well as in grey eyes.

No albinos were met with in the material examined by me, and my observations, therefore, relate to pigmented eyes only.

It has generally been supposed that the various types of eye-colours grade into each other without sensible breaks of continuity. A critical examination, however, shows that there is a distinct discontinuity between:—

(1) The eyes in which two kinds of pigments are present; the one, yellow-brown in colour, deposited on the outer or anterior surface of the iris; the other, blue-black in colour, deposited on the inner or posterior surface of the iris. Such eyes I propose to call *duplex.*

(2) The eyes in which the posterior pigment alone is present in the iris, the anterior pigment being absent. Such eyes may be called *simplex.* The application of popular names to these types is uncertain and quite unreliable; but, in general, eyes that would be called brown belong to the duplex type, while many of the blues and some of the greys belong to the simplex type.

(1) *The Duplex Type.*

To the duplex type belong the various shades of eyes with both anterior and posterior pigments.

In my observations, three distinct patterns of duplex eyes were found, viz.:—

(*a*) The *self-coloured* duplex, in which the anterior pigment is distributed over the whole front of the iris, practically obscuring the posterior pigment as in ordinary brown eyes.

(*b*) The *ringed* duplex, in which the anterior pigment is confined to a ringed area round the pupil, leaving the ground colour of posterior pigment clearly exposed round the periphery of the iris.

(*c*) The *spotted* duplex, in which the anterior pigment is broken up into distinct blotches or spots irregularly scattered over the posterior pigment which forms the ground colour.

Self-coloured duplex eyes vary in shade, presenting different grades of anterior pigmentation. In the darker brown shades the anterior pigment is more densely deposited than in the lighter green shades, the green effect being produced by a thin layer—or in some cases merely a fine irroration—of yellow pigment above the blue posterior layer.

Ringed duplex eyes similarly vary in shade, presenting different grades of anterior pigmentation. In my experience, the distribution of anterior pigment is always denser immediately round the pupil, both in self-coloured and ringed duplex eyes. Eyes with a ring of anterior pigment round the periphery of the iris, but without anterior pigment round the pupil, were not found in my observations.

Spotted duplex eyes also present different grades of anterior pigmentation. The pigmented areas also vary in size and number in different individuals and in the two eyes of the same individual.

Low-grade forms of the ringed duplex pattern are apparently numerous: under ordinary observation these might no doubt pass as blue or grey simplex eyes, the small pigmented ring so blending with the dark pupil as to be unrecognised at a short distance.

Similarly, low-grade forms of the spotted duplex pattern would also pass as blue or grey simplex eyes at a short distance. In both cases, however, a closer inspection reveals their true nature.

In carrying out my observations, I found that about half of the eyes, which appeared to be simplex when viewed in the ordinary way, were really duplex when closely examined in a good light.

Eyes presenting grades of anterior pigmentation so low that they can be mistaken for simplices have not occurred in my experience, and if such eyes do occur, they must be extremely rare in the population which I have studied.

In duplex eyes the anterior pigment is visible soon after birth, so that quite young infants are included in my observations. That anterior pigmentation tends to increase with age in young children is evident from the results of my observations during three years, though to what extent is not yet clear, owing to the limited period of observation.

(2) *The Simplex Type.*

To the simplex type belong the various shades of eyes with posterior pigment only, the anterior pigment being quite absent, as in all clear blue and clear grey eyes.

The darker shades of blue are apparently due to the greater delicacy and transparency of the fibrous tissues of the iris through which the posterior pigment is seen, while the lighter shades of blue and the coarser greys seem to be due to the greater coarseness and opacity of the same tissues.

These structural differences appear to be continuous, a series of intermediate blue-greys linking up the finer blues with the coarser greys.

A further complication arises from the fact that, in most cases, the finer tissues of the iris become coarser with age, and young children with dark blue eyes may mature into adults with light blue, blue-grey, or grey eyes.

I have found striking illustrations of this in large families, where a whole series of shades from blue to grey were seen, the younger children being blue and the older ones grey. Consequently, no attempt has been made to distinguish between the many shades of clear blue and clear grey eyes, but all eyes with posterior pigment only are classed as simplex.

Finally, the clear blue and clear grey simplex eyes, with no trace of anterior pigment, must be carefully distinguished from the numerous low-grade duplex eyes, previously noted, which are often called "blue" or "grey."

Heredity of the Duplex and Simplex Types.

All families with less than two children are omitted, and families of two were not taken freely. At first, the largest families available were selected, but afterwards it seemed desirable to study collaterals, consequently some small families are included.

Out of the 139 families observed, 20 were simplex matings, 50 were duplex matings, and 69 were duplex-simplex matings.

Simplex Matings.

The 20 matings of simplex females with simplex males produced 101 offspring, all of the simplex type. Table I gives the numbers found in each family. Each family is distinguished by the number and initials of the male parent:—

Table I.—Simplex × Simplex.

Number and initials of ♂ parent.	Duplex.	Simplex.	Total offspring.
15 A. F.	0	4	4
27 J. T.	0	12	12
31 D. N.	0	11	11
89 J. M.	0	3	3
91 S. H.	0	3	3
93 F. G.	0	12	12
97 H. C.	0	6	6
101 J. L.	0	7	7
105 T. S.	0	4	4
113 E. D.	0	2	2
133 W. D.	0	4	4
137 J. C.	0	7	7
217 J. A.	0	4	4
221 J. D.	0	2	2
231 W. K.	0	5	5
235 A. B.	0	7	7
243 J. F.	0	2	2
255 J. H.	0	2	2
257 J. C.	0	2	2
277 A. A.	0	2	2
20	0	101	101

Duplex Matings.

The 50 matings of duplex females with duplex males gave two kinds of results: (*a*) 37 families produced 195 offspring, all of the duplex type; (*b*) 13 families produced 63 offspring, of which 45 were duplex and 18 simplex. Tables II (*a*) and (*b*) give the numbers found in each family.

Table II.—Duplex × Duplex.

No. and initials of ♂ parent.	Duplex.	Simplex.	Total offspring.
(*a*) Giving all Duplex.			
5 W. H.	8	0	8
13 J. C.	2	0	2
33 T. S.	8	0	8
39 J. P.	6	0	6
41 W. H.	7	0	7
43 G. L.	6	0	6
55 J. B.	10	0	10
57 J. B.	3	0	3
59 C. B.	12	0	12
65 J. F.	7	0	7
69 B. H.	5	0	5
71 R. E.	6	0	6
73 A. S.	6	0	6
81 T. C.	9	0	9
83 J. W.	3	0	3
85 W. P.	4	0	4
103 H. E.	5	0	5
117 C. H.	6	0	6
123 J. S.	4	0	4
129 J. W.	8	0	8
131 J. G.	6	0	6
135 T. W.	4	0	4
139 J. B.	3	0	3
141 M. C.	2	0	2
145 W. P.	2	0	2
161 J. M.	5	0	5
173 B. B.	10	0	10
177 F. S.	2	0	2
191 E. G.	4	0	4
211 T. R.	2	0	2
215 C. W.	3	0	3
223 H. G.	4	0	4
237 G. K.	4	0	4
259 G. G.	3	0	3
265 J. R.	5	0	5
269 W. P.	3	0	3
275 A. P.	8	0	8
37	195	0	195
(*b*) Giving Duplex and Simplex.			
107 W. S.	1	1	2
111 J. W.	2	1	3
115 T. B.	3	1	4
165 E. E.	5	1	6
169 G. B.	5	1	6
219 J. R.	5	1	6
227 E. H.	2	1	3
249 H. H.	6	2	8
253 A. F.	3	2	5
261 J. M.	4	2	6
263 A. N.	3	3	6
267 E. D.	3	1	4
271 W. W.	3	1	4
13	45	18	63

Duplex-Simplex Matings.

The 69 matings of duplex and simplex parents also gave two kinds of results: (*a*) 17 families produced 66 offspring, all of the duplex type; (*b*) 52 families produced 258 offspring, of which 121 were duplex and 137 were simplex.

Table III (*a*) and (*b*) give the numbers found in each family.

Table III.—Duplex × Simplex.

Number and initials of ♂ parent.	Duplex.	Simplex.	Total offspring.
(*a*) Giving all Duplex.			
9 S. E.	2	0	2
45 J. H.	8	0	8
53 D. A.	2	0	2
67 J. H.	9	0	9
77 M. G.	3	0	3
121 L. C.	4	0	4
125 E. R.	3	0	3
143 W. D.	4	0	4
147 W. B.	2	0	2
167 T. C.	7	0	7
181 J. B.	5	0	5
201 E. A.	3	0	3
205 W. B.	4	0	4
209 J. H.	2	0	2
213 J. K.	2	0	2
239 H. C.	3	0	3
245 J. T.	3	0	3
17	66	0	66

Table III—*continued.*

Number and initials of ♂ parent.	Duplex.	Simplex.	Total offspring.
(*b*) Giving Duplex and Simplex.			
1 C. H.	3	2	5
3 G. F.	0	6	6
7 W. E.	3	5	8
17 W. J.	1	1	2
19 F. B.	3	1	4
21 J. B.	4	5	9
23 T. P.	0	4	4
25 R. B.	2	3	5
29 A. F.	5	5	10
35 R. C.	3	4	7
37 S. E.	4	2	6
47 J. S.	3	3	6
49 T. K.	2	2	4
51 G. D.	1	2	3
61 T. C.	5	1	6
63 T. L.	4	5	9
75 J. A.	2	3	5
79 W. W.	4	3	7
87 J. B.	5	4	9
95 J. R.	4	5	9
99 C. C.	5	4	9
109 W. P.	1	1	2
119 J. T.	2	4	6
127 G. S.	3	1	4
149 H. T.	1	4	5
151 W. R.	5	2	7
153 J. M.	3	3	6
155 W. N.	1	1	2
157 T. L.	3	3	6
159 W. H.	4	1	5
163 W. H.	3	3	6
171 D. S.	4	3	7
175 J. C.	2	3	5
179 G. G.	1	1	2
183 W. E.	1	1	2
185 R. S.	2	3	5
187 R. A.	1	1	2
189 G. K.	2	4	6
193 T. C.	1	2	3
195 J. P.	3	1	4
197 J. P.	1	1	2
203 A. P.	1	2	3
207 J. L.	1	1	2
225 C. H.	3	1	4
229 J. N.	1	2	3
233 J. B.	0	4	4
241 J. R.	2	3	5
243 J. F.	2	1	3
247 J. H.	0	2	2
251 A. B.	2	3	5
273 J. P.	2	1	3
279 E. C.	0	4	4
52	121	137	258

With regard to the heredity of the duplex and simplex types, the above tables show that:—

(1) Simplex parents mated together give all simplex offspring.

(2) Duplex parents mated together give either (*a*) all duplex offspring, or (*b*) duplex and simplex offspring in the proportion of about 3 : 1.

(3) Duplex parents mated with simplex parents give either (*a*) all duplex offspring, or (*b*) duplex and simplex offspring in the proportion of about 1 : 1. It is evident, therefore, that the simplex type, in heredity, behaves as a Mendelian recessive to the duplex type, which is dominant. We have already seen that the duplex type differs from the simplex type by the presence of anterior pigment in the iris.

The unit-characters concerned in the heredity of the duplex and simplex types of eyes are, therefore, presence (duplex) and absence (simplex) of anterior pigment in the iris, presence being dominant over absence, which is recessive.

The duplex parents in Tables II (*b*) and III (*b*) are all obviously heterozygous, carrying the simplex character in a recessive state.

The duplex parents in Tables II (*a*) and III (*a*) may be either homozygous or heterozygous.

The duplex parents in Table III (*a*) with large families are almost certainly homozygous, but in those with small families the numbers of offspring are insufficient to test adequately the gametic constitution of the duplex parents. Similarly in the case of the large families in Table II (*a*), one of the duplex parents is almost certainly homozygous, while the other parent may be either homozygous or heterozygous.

In the small families the numbers of offspring are again too small to test adequately the gametic constitution of the duplex parents.

In proof of this we have the fact that in Table III (*a*) each of the three duplex parents of families 53 D. A., 125 E. R., and 209 J. H. is known to have a simplex parent and consequently must be heterozygous, though none of them has yet produced simplex offspring. Similarly in Table II (*a*) both duplex parents of family 13 J. C. are known to have a simplex parent, though they have not yet produced any simplex offspring. As might be expected in a mixed population, some of the simplex parents observed are known to have been extracted from a duplex parent, or to have had duplex brethren.

For instance, in Table I, at least one of the simplex parents of families 15 A. F., 97 H. C., 231 W. K., 255 J. H., and 257 J. C. is known to have a duplex parent, while of the families 31 D. N. and 243 J. F. one parent at least has duplex brethren.

In accordance with the Mendelian principles, the extracted simplex type breeds true to the simplex character without reversion to its duplex ancestors. In view of recent Mendelian experiments with plants and animals, it did not seem impossible that some simplex individuals at least might be carrying factors which on meeting, in the process of fertilisation, with other complementary factors, might give rise to reversions of the duplex type, but, so far, no such cases have been found. For the present, therefore, while remembering such a possibility, we must take it that the duplex and simplex types of eye-colour in man constitute a simple Mendelian case of presence and absence of a certain pigment.

With regard to the different patterns of duplex eyes and their various shades, my facts, so far, do not enable me to determine positively their genetic relations. The chief difficulty is due to the fact that the anterior pigment present in children tends to increase with age, though to what extent, or to what age, is not yet known, owing to the limited period of observation.

In the few families observed, with all adult offspring, the evidence suggests that the ringed pattern is recessive to the self-coloured pattern, which is dominant; but with regard to the genetic relations of the spotted pattern to the ringed and self-coloured patterns there is practically no evidence available.

Previous Work.

Large numbers of records of eye-colours have been compiled and discussed by anthropologists and biometricians at home and abroad. In most cases, however, the data relate to certain sections of the population, such as school children and conscripts, not analysed or grouped according to their families. So far, I have found only two memoirs which approach the question of the inheritance of eye-colour in man by a comparison of parents with their offspring. The first is that of Alphonse de Candolle.*

De Candolle, with the assistance of some 28 experienced observers, collected a number of records of the inheritance of eye-colours in Switzerland, Germany, and Sweden. De Candolle made two classes of eye-colours, "brown" and "blue," omitting all doubtful shades. The "brown" class included "black," "brown," "yellow-brown," and "green-brown." The "blue" class included "blue," "blue-grey," "grey," "green-blue," and "green-grey." Comparing de Candolle's classes and shades with mine, it is evident that all the "brown" class belong to the duplex type with anterior pigment, but that only part of the "blue" class belong to the simplex type, without anterior pigment.

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* "Hérédité de la Couleur des Yeux dans l'Espèce Humaine," 'Archives des Sciences, Genève (3ème période, vol. 12, 1884, pp. 97—119).

The second memoir is that of Mr. Francis Galton.* Mr. Galton collected a number of records of family eye-colours in the British Isles, among the "Records of Family Faculties"—known as the R.F.F. data. These family records were obtained through the offer of prizes to the public.†

From the family records sent in, Mr. Galton made three classes of eye-colours, "light," "hazel," and "dark." The "light" class included the shades recorded as "light blue," "blue," "dark blue," "grey," and "blue-green." The "hazel" class included "dark grey" and "hazel." The "dark" class included "black," "very dark brown," "dark brown," "brown," and "light brown." Comparing the shades of colour sent in to Mr. Galton by his correspondents with mine, it is evident that all of Mr. Galton's "dark" class belong to the duplex type with anterior pigment. With regard to the "hazel" class, part of these would probably represent my ringed duplex pattern, while the remainder might belong either to the duplex or simplex type, according to the interpretations of the colours by different observers. Mr. Galton apparently regards "dark grey" and "hazel" as bicolour eyes,‡ which would make them practically equivalent to my ringed duplex eyes. In view, however, of my experience with popular descriptions of eye-colours, it is highly probable that many of Mr. Galton's correspondents would record certain forms of self-coloured duplex eyes as "hazel," and certain forms of "simplex" eyes as "dark grey." Mr. Galton's "light" class would apparently consist partly of the simplex type without anterior pigment, and partly of the low-grade forms of the duplex type with some anterior pigment. In the nature of the circumstances in which the R.F.F. data were recorded, it cannot, of course, be expected that the observations were critical in regard to the presence or absence of anterior pigment in the iris. On the whole, therefore, it does not seem possible to express either de Candolle's or Mr. Galton's classes and shades of eye-colours in terms of duplex and simplex pigmentation. It was on Mr. Galton's R.F.F. data that Professor Karl Pearson based his memoir "On the Inheritance of Eye-colour in Man,"§ and afterwards concluded that nothing corresponding to Mendel's principles appeared in the characters for eye-colour in man.||

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* "Family Likeness in Eye-colour," 'Roy. Soc. Proc.,' 1886, vol. 40, No. 245, pp. 402—416.

† See 'Natural Inheritance,' 1889, pp. 72—78.

‡ *Loc. cit.*, pp. 142, 144.

§ 'Phil. Trans.,' A, 1900, vol. 195, p. 102.

|| 'Biometrika,' 1903, vol. 2, pp. 213, 214.

Summary.

An examination of the eye-colours of a number of parents and their offspring in a Leicestershire village shows that there are at least two discontinuous types of iris in man:—

(1) The duplex type, with both anterior and posterior pigments, as in ordinary brown eyes.

(2) The simplex type, with posterior pigment only, the anterior pigment being absent, as in clear blue eyes.

In heredity the simplex type behaves as a Mendelian recessive to the duplex type, which is dominant. The unit characters concerned are evidently presence (duplex) and absence (simplex) of anterior pigment on a basis of posterior pigment, presence being dominant.

The duplex and simplex types can be distinguished at any age. Various pigmental and structural changes take place in the iris during childhood and youth, the extent of which is not yet known. Few families with living parents and offspring, all adult, are to be found in one village. Consequently, it has not yet been possible to determine the genetic relations between the various shades of the duplex type.

Note.—I desire to acknowledge my indebtedness to Mr. W. Bateson for some valuable criticisms and suggestions in regard to the preparation of this paper, and also to Mr. R. C. Punnett, who came down and examined a number of the simplex eyes recorded above.

(*Note added* 11*th January*, 1908.)

Since the above paper was presented, an article on "Heredity of Eye-colour in Man" has appeared in 'Science,' 1907, vol. 26, pp. 589—592 (dated 1st November), in which Professor C. B. Davenport independently arrives at similar conclusions, pointing out the Mendelian inheritance of eye-colour in man.

On Some Features in the Hereditary Transmission of the Self-black and the "Irish" Coat Characters in Rats.—Paper I.

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A. Introductory.

In 1903 I commenced a series of breeding experiments with rats. They were undertaken with the object of ascertaining whether Mendel's Principles would adequately interpret the observed facts.

During the interval of four years which has since elapsed, many records of results have been made. But a consideration of these initial results shows that the problems involved are of some complexity, and many years of continuous work will be needed before the hypothetical assumptions, which tentatively have had to be adopted, can be regarded as proved. Notwithstanding this, it can be shown by the whole of the results so far reached—only part of which are here recorded—that the phenomena may be explained on the basis of segregation, gametic purity, and of dominance, if we tentatively accept certain postulates which are enumerated below (*infra*, p. 99).

I purpose describing my results in a series of separate papers, of which this is the first.

At the time that my experiments were undertaken, the only papers which had been published on the hereditary transmission of coat colours in rats were those of Crampe (13 and 14), published in 1877, 1883, 1884, and 1885. All his papers were therefore published antecedent to the re-discovery of Mendel's Principles, by De Vries, in 1900, and the results were not stated in a form which render it possible to determine certainly whether they are in accordance with Mendelian expectations or not. Crampe's experiments were analysed by Bateson in 1903 (5), and he showed that a few Mendelian conclusions could be deduced from them.

But at about the same time that I commenced my experiments, Doncaster had also instituted a similar series with the same kind of animals, and in 1905 he published his results (15).

He also discussed Crampe's experiments, and as the outcome of his own work he reached the following conclusions:—(1) That albinoes may carry the determiners for grey and for black, or for both. That some may carry the piebald determiners, and others the self pattern determiners. (2) That Crampe's "Irish" form consisted of two sub-forms, which Doncaster designated "*a*" and "*b*." (3) That the "Irish" "*a*" and the wholly black form Cr 7, contained no piebald. (4) That, tentatively, the Cr 7 (wholly black) form may be regarded as an extreme form of the Cr 6 *a*, and is not a separate variety. (5) That with regard to pattern there are only two allelomorphs in rats, *i.e.*, "self" and "piebald," and that when they are crossed together, dominance is not complete. (6) That the "Irish" *b* form is a heterozygote produced by crossing black (Cr 7) or "Irish" *a* with black and white. (7) That the proportions of types which appear in successive litters may be diverse.

With regard to these conclusions, my work confirms them all, with the exception of No. 4, and it adds further evidence in support of most of them. In addition, some new results are reached. With respect to these latter and to the extended evidence, see "Summary of Results," p. 115.

I have to express my thanks to Mrs. Gunn (Miss Gertrude Smith), who for three years daily superintended the feeding of the rats. To her conscientious care must be ascribed much of the success of the breeding.

My thanks are also due to Mrs. F. Judkins, of Sark, who personally took considerable trouble to obtain for me three pairs of the old English black rat (*Mus rattus*). Unfortunately, this species apparently will not breed with the grey rat (*Mus decumanus*), nor with any of the tame types. I have kept *M. rattus* mated with all the varieties for considerable periods, but without result.

To Mr. Reginald I. Pocock, I am also indebted for two specimens of the black *variety* of the wild grey rat, which were trapped in the Zoological Gardens, and for five young specimens of *M. rattus*, which were born there, from a pair presented by Mrs. Judkins.

The cost of these experiments has been defrayed by a grant from the Government Grant Committee of the Royal Society.

B. THE SYSTEM OF INTERPRETATION OF RESULTS.

It is possible to adopt at least three ways of representing the allelomorphic pairs, but so far as the results which I have obtained with rats are concerned, the predictions are identical in each case.

One of these "schemes" differs fundamentally from the other two in that it regards greyness as not being allelomorphic to blackness, while the other two do. There are reasons for believing that greyness and blackness may not be allelomorphic. And since this "scheme," which embodies Bateson and Punnett's "Presence and Absence" theory, has successfully interpreted other results of a diverse nature, it will be adopted in this paper.

The essential feature in their "scheme" is that the allelomorphic pairs consist of a determinant (factor) for the presence, and of one for the absence of the character under consideration. On the basis of gametic purity, therefore, any one gamete cannot carry both the positive and negative factor of the same character. But it can carry the positive factor for each of two characters, such as blackness and greyness.

This "scheme" predicts accurately the types of rat which result in any cross, in so far that types not predicted never appear. In some cases, however, predicted types have not appeared, but the evidence is clear that this is due only to want of numbers, and that with more litters the missing types will duly appear. Indeed, in several instances, when I first tabulated my results, a certain type which was predicted was absent, but in most of the cases it has since appeared in a later litter. With regard to the expected proportions, the prediction is usually approximately, and in some cases exactly, fulfilled.

In interpreting my results by means of Bateson and Punnett's "Presence and Absence" hypothesis, I have conjoined to their conception, that of Cuênot, in which he postulates the idea that colour is due to the interaction of two bodies, one a ferment and the other a chromogen. This idea has received confirmation from Miss Durham's work.

Below I append the various symbols employed :—

C = colour producer.*	c = absence of colour producer.
G = grey determiner.†	g = „ grey determiner.
B = black† „	b = „ black „
S = self-pattern determiner.	s = „ self-pattern determiner.
P = piebald-pattern determiner.	p = „ piebald-pattern determiner.

---

* In this paper the colour producer is regarded as a ferment.

† In this paper the grey and black determiners are regarded as chromogenous bodies which develope colour when acted upon by the ferment C.

The different types of rats will therefore be symbolically represented as follows :—

Wild self-grey rat......	CGBSP.
Piebald „	CGBsP.
Wild self-black rat ...	CgBSP.
Piebald „ ...	CgBsP.
Albino rat..............	cGBSP, cGBsP, cgBSp, cGbSp, cGbSP, cgBSP, cGbsP, cgBsP, cGBSp.

By various combinations of these forms the number of possible albinoes can be increased. Thus, if cgBSP × cGBSp, a form cGgBSPp will result.

It is very doubtful whether an albino not carrying at least one positive colour and one positive pattern member of each allelomorphic pair, exists.

In accordance with the indications given by Miss Durham's work (17), I shall assume that the albino carries the chromogen determiners (G and B) and not the ferment = tyrosinase = colour producer (C).

In applying this "scheme" to my results, it will be necessary to assume that every gamete must carry one member of each allelomorphic pair.

C. THE "IRISH" CHARACTER = CRAMPE'S CR 6.*

The form known in the "fancy" as the "Irish" rat is a nearly wholly black individual with patches of white on the ventral surface. With regard to its visible zygotic characters there are apparently two types which Doncaster (15) has proposed to call the "Irish" *a* and the "Irish" *b*. With this subdivision of the "Irish" type (Cr 6) I am inclined to agree, and certainly for purposes of description it is a very necessary one.

Doncaster's definition of the zygotic characters of the "*b*" form is, however, unsatisfactory, because it is not sufficiently precise. According to him this form has more white on the ventral surface than the "*a*" form. My experience suggests that this is not the chief difference, and that we should define the "Irish" *b* as being the form with more or less ventral white and *with carpal and metatarsal white bands.* The "Irish" *a* possesses the ventral white alone and always very little of it, but has no carpal or metatarsal bands; the ventral white is usually merely a fleck on the chest or abdomen. Some of my "Irish" *b* forms in respect of the excessive reduction of the ventral white approach the *a* form, but are at once distinguished by the presence of the white carpal and metatarsal bands.

---

* The symbols Cr 1—Cr 7, which are used in this paper, indicate the seven varieties into which Crampe divided rats. These varieties are described on p. 119, and also by Bateson (5), and by Doncaster (15).

As a rule, the ventral white in the "Irish" *b* form assumes the character of a longitudinal band extending from the chest at the level of the arms back to the umbilicus or farther. In general form, this band is narrow-triangular with its base in the pectoral region. In most cases, the two angles of the base extend off along the ventral surface of the arms and reach the carpal bands. In some instances, however, this extension is absent, and in others broken. The longitudinal band itself is often broken, or expanded in some parts, usually in the abdominal region, and constricted in others, usually just anterior to the umbilical region. In the pattern thus produced there is often symmetry, but usually more or less departure from a strict symmetrical figure exists.

In the "Irish" *a* form, the ventral white in most cases assumes the shape of a very small triangular patch in the pectoral region, with its apex directed posteriorly. It suggests a reduced pattern of the "Irish" *b* form. Sometimes an additional spot of white is present on the abdomen.

Origin of the "Irish" b.

The "Irish" *b* is a heterozygote and in my experiments has been produced in the following 10 different kinds of crosses:—

(1) Black var. of wild grey (Cr 7) × Cr 4.*
(2) Extracted black (Cr 7) × Cr 4.
(3) Cr 2 × Cr 2.†
(4) Cr 2 × Cr 4.
(5) Cr 2 × Cr 5 (carrying albinism recessive).
(6) Cr 5 × Cr 4.
(7) "Irish" *b* × "Irish" *b* (Cr 6 *b*).
(8) "Irish" *b* × Cr 5 (homozygote).
(9) "Irish" *b* × Cr 5 (carrying albinism recessive).
(10) "Irish" *b* × Cr 4.

Of these, Doncaster has previously obtained "Irish" *b* from Nos. 6 and 10, and also probably from crosses Nos. 2, 3, 4, 5, and 7. In these latter crosses Doncaster had not, at the time they were made, distinguished between "Irish" *b* and *a*. But in the light of my experiments there can be no doubt his "Irish" forms from these crosses were of the sub-type *b*. The origin of "Irish" *b* forms from crosses Nos. 1, 8, and 9, are, therefore, new. In addition to these, Doncaster also obtained this form from crosses of Cr 6 *a* × Cr 6 *b*, Cr 7 × Cr 5, and Cr 7 × Cr 6 *b*. It is almost certain from his records, that the Cr 7 in the two latter experiments (53 and 34) was not homozygote and was, perhaps, an "Irish" *a* (see Summary of Results, No. 5, p. 115).

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* See footnote, p. 100.

† The Cr 2 forms are similar in their visible zygotic characters to the "Irish" *b*, but are grey in colour.

The Cr 2* types used in my experiments, and also the "Irish" *b*, were of two different origins, one form in each type resulting from a cross of a self-grey or a self-black respectively with a Cr 4, and in the other with a corresponding cross with a Cr 5. The possibility that the Cr 4 in the two former cases carried the piebald pattern determiner must not be overlooked. The homozygote nature of the Cr 5 in the eighth line of the column of matings above was tested by crossing it with a Cr 5 known to be carrying albinism recessive. No albinoes appeared in its offspring.

D. Experimental Results.

(1) "*Irish*" *b* × "*Irish*" *b* = *Cr* 6 *b* × *Cr* 6 *b*.

(a) *Albino Parentage.*—Seven matings of this kind have been made. Of the seven pairs of "Irish" *b* individuals used, two pairs (experiments 49, 50) were derived from a cross of a black var.: wild grey × albino, three (51, 52, 53) from a cross of an extracted black (Cr 7) × Cr 4, and two (experiments 54, 55) from a cross of "Irish" *b* × Cr 5 carrying albinism recessive.

The black variety of the wild grey rat may be regarded as a pure black and as equivalent to an extracted Cr 7. There is no possibility of its carrying the grey chromogen determiner, since grey is dominant to black. We may, therefore, consider experiments 49, 50, 51, 52, and 53 together and regard them all as crosses of a Cr 7 × Cr 4.

The parental albinoes of these "Irish" *b* individuals were bred by me from albino grandparents, which were in turn obtained from ordinary dealers. Rats obtained from this source are nearly sure to be of piebald origin, though one of mine thus obtained for another experiment carried the self character, and another the grey chromogen. We may, therefore, assume that these albinoes were carrying the piebald pattern and black chromogen determiners.

On this assumption we may represent the "Irish" *b* individuals used by the symbol CcgBSsP.

It will, perhaps, be as well in this particular cross to show how the predictions which are deduced are arrived at, and I will therefore give a table of the possible gametic combinations.

Each "Irish" *b* individual of the zygotic composition CcgBSsP will, at the segregation of the characters, form gametes which will carry the combination of characters shown in the following symbols: CgBSP, CgBsP, cgBSP, cgBsP. There are thus 16 possible combinations between the four kinds of gametes. The combinations are shown in the subjoined Table I.

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* See footnote, p. 100.

Table I.—Predicted Offspring in a Cross of "Irish" *b* × "Irish" *b*.

Gametes formed by "Irish" *b*.		Gametes formed by "Irish" *b*.		Zygotic composition of the offspring.		Visible zygotic characters of the offspring.
CgBSP	×	CgBSP	=	CgBSP	=	Cr 7
CgBsP	×	CgBSP	=	CgBSsP	=	Cr 6 *b*
cgBSP	×	CgBSP	=	CcgBSP	=	Cr 6 *a*
cgBsP	×	CgBSP	=	CcgBSsP	=	Cr 6 *b*
CgBSP	×	CgBsP	=	CgBSsP	=	Cr 6 *b*
CgBsP	×	CgBsP	=	CgBsP	=	Cr 5
cgBSP	×	CgBsP	=	CcgBSsP	=	Cr 6 *b*
cgBsP	×	CgBsP	=	CcgBsP	=	Cr 5
CgBSP	×	cgBSP	=	CcgBSP	=	Cr 6 *a*
CgBsP	×	cgBSP	=	CcgBSsP	=	Cr 6 *b*
cgBSP	×	cgBSP	=	cgBSP	=	Cr 4
cgBsP	×	cgBSP	=	cgBSsP	=	Cr 4
CgBSP	×	cgBsP	=	CcgBSsP	=	Cr 6 *b*
CgBsP	×	cgBsP	=	CcgBsP	=	Cr 5
cgBSP	×	cgBsP	=	cgBSsP	=	Cr 4
cgBsP	×	cgBsP	=	cgBsP	=	Cr 4

$$\text{Total offspring} = 4\ \text{Cr}\ 4 + \underbrace{3\ \text{Cr}\ 5 + 2\ \text{Cr}\ 6\,a + 6\ \text{Cr}\ 6\,b + 1\ \text{Cr}\ 7.}$$

4 alb. : 12 pigmented.
= 1 : 3

Turning now to the actual results of experiments 49, 50, 51, 52, and 53, there is produced a total offspring of:—

$$9\ \text{Cr}\ 4 + \underbrace{4\ \text{Cr}\ 5 + 3\ \text{Cr}\ 6\,a + 10\ \text{Cr}\ 6\,b + 4\ \text{Cr}\ 7.}$$

= 9 alb. : 21 pigmented.

Thus the experimental result departs from prediction in the expected proportions only. Dividing the actual result throughout by two, we have 4·5 + 2 + 1·5 + 5 + 2. This is a very close approximation to the prediction. The actual result is only one Cr 5 and Cr 6 *b* too few, and one Cr 7 too many.

It is instructive to plot out the prediction and the actual result in the form of two curves (fig. 1). It can be seen at once how close is the resemblance between the two. It is obvious that the general slope and form is the same, and that the difference is not greater than that between a theoretical normal curve of error, and one of that form obtained from an actual series of measurements.

It is to be noted that all the types which are predicted have appeared, and that none which are not expected have arisen.

We may, therefore, conclude that the "Irish" *b* form in these experiments may have the gametic composition which I have assumed for it, *i.e.*, CcgBSsP.

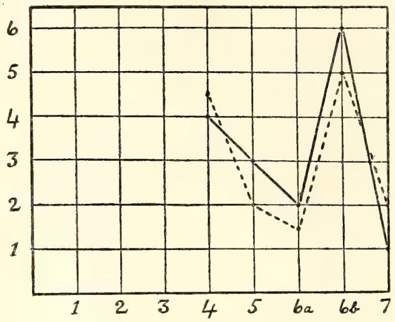

Fig. 1.—Curve showing the degree of correspondence between prediction and actual result in a cross of "Irish" *b* × "Irish" *b*. The line indicating prediction is the continuous one, that showing result is the broken one. The abscissæ represent the different types of rats, and the ordinates the proportions in which they occur.

(b) *Piebald Parentage.*—In experiments 54 and 55 the "Irish" *b* parents used were derived from a cross of Cr 6 *b* × Cr 5 (carrying albinism recessive). The Cr 6 *b* grandparent was in both experiments derived from a cross of black variety of wild grey × Cr 4 (see experiments 47, 57, and 59). From such a cross of Cr 6 *b* × Cr 5, there are expected among the offspring, in addition to the Cr 4 and Cr 5 types, two forms of "Irish" *b*, *i.e.*, CgBSsP and CcgBSsP.

If we suppose that in both of the experiments (54 and 55) one of the "Irish" *b* parents was a CgBSsP and the other a CcgBSsP, then the predicted results will differ from that where both are CcgBSsP in the absence of albinoes. The expectation is:—

2 Cr 5 + 1 Cr 6 *a* + 4 Cr 6 *b* + 1 Cr 7.

The actual result of the two experiments combined is:—

3 Cr 5 + 8 Cr 6 *b* + 1 Cr 7.

The special feature of the prediction, *i.e.*, absence of albinoes, is thus far confirmed. Considering the smallness of the number, the approximation to prediction, with regard to proportion of the different types, is sufficiently close to be significant. The proportion of Cr 5 is exact, that of the Cr 7 is as near as it can be with the number of individuals present, while that of the Cr 6 *b* is two too many. The predicted Cr 6 *a* is absent.

In connection with the missing type, the remark made on pp. 99 and 106 should be borne in mind. Expected types do not always appear in the first litter, and some not until the third litter, even though the early litters may be of large numbers (eight or more).

We may, therefore, regard these two experiments as affording initial evidence that this particular type of "Irish" *b*, *i.e.*, CgBSsP as contrasted to CcgBSsP has been actually produced.

(2) *"Irish" b × Piebald Black-White = Cr* 6 *b* × *Cr* 5.

Five matings of this nature have been made (experiments 56, 57, 58, 59, and 59*a*). In experiment 56 the Cr 5 parent was known by two previous testings to be homozygote. In experiments 57 and 59 the Cr 5 parent was known from previous testings to be carrying albinism recessive. In experiment 58 the Cr 5 parent was not tested, but since both its parents carried albinism recessive, the chances are equal that it also carried albinism. In experiment 59*a* the chances are also equal that the Cr 5 parent is either homozygous or carries albinism recessive.

The "Irish" *b* partners were in four experiments (56—59) members of the same parents, and were derived from a cross between a black variety of wild grey × Cr 4 (experiment 47). The albino parent was derived from albino grandparents purchased from ordinary dealers, and it was therefore, in all probability, carrying both black chromogen and piebald pattern determiners.

The "Irish" *b* partners in these four experiments may be, therefore, represented by the symbol CcgBSsP. The piebald parents in experiments 57, 58, and 59 will be represented by the symbol CcgBsP.

The predicted offspring in such a cross will be :—

$$2\ \mathrm{Cr}\ 4+\underbrace{3\ \mathrm{Cr}\ 5+3\ \mathrm{Cr}\ 6\ b.}_{}$$

2 alb. : 6 pigmented. = 1 : 3.

The actual result for experiments 57 and 59 combined is :—

$$12\ \mathrm{Cr}\ 4+\underbrace{12\ \mathrm{Cr}\ 5+20\ \mathrm{Cr}\ 6\ b+2\ \text{pig. eyes.*}}_{}$$

12 alb. : 34 pigmented. = 1 : 3 nearly.

In these two experiments (57 and 59) where the actual gametic factors are known with some degree of certainty, the prediction of proportions of Cr 4 to the pigmented types and the actual result are practically identical. The proportion, however, of Cr 6 *b* is a little too large.

In the case of experiment 57 there were five litters, and since the details are significant, I append them :—

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[*] At birth the pigmented individuals can be immediately distinguished from the albinoes by the pigmented eyes, which show through the skin. These two young ones died before their coat colour and pattern had developed. At birth the skin of all rats is colourless and hairless.

1st. 2 Cr 4 +6 Cr 6 *b*.
2nd. 2 Cr 4+3 Cr 5+2 Cr 6 *b*.
3rd. 3 Cr 4+2 Cr 5+2 Cr 6 *b*+1 pigmented eyes.
4th. 3 Cr 4+2 Cr 5+2 Cr 6 *b*.
5th. 2 Cr 4+1 Cr 5+3 Cr 6 *b*+1 pigmented eyes.
12 8 15 2

It is to be noted in the first litter, notwithstanding that it is large, that the predicted Cr 5 type is not present. That in the first, second, and third litters the proportion of albinoes to pigmented individuals is as near as it possibly can be to expectation, with the odd numbers involved. That in the third and fourth litters the albinoes are in excess.

These considerations show us, what is also shown in others of my experiments, that predicted types will not necessarily appear in any one litter, and that large numbers must be reared before expected proportions in all cases will be quite accurately realised by actual results.

Considering next, experiment 56, where the Cr 5 parent is known to be homozygote, the prediction is :—

2 Cr 5+2 Cr 6 *b*.

The actual result is :—

4 Cr 5 : 3 Cr 6 *b*.

With the odd numbers concerned this is the nearest approach possible to prediction. The chief feature to notice is that while the predicted types have appeared, unpredicted ones have not.

Experiments 58 and 59*a* may now be considered. The absence of albinoes in their offspring suggests that the Cr 5 parent was homozygous. The total offspring in these two experiments is 4 Cr 5+8 Cr 6 *b*. Added to experiment 56, it gives a total of 8 : 11 respectively. There is thus, roundly, only one Cr 5 too few, and one Cr 6 *b* too many.

(3) *"Irish" b* × *Albino* = *Cr* 6 *b* × *Cr* 4.

Seven crosses of this nature have been made, *i.e.*, experiments 60 to 66. All the "Irish" *b* parents used were descended from two individuals of the black variety wild grey rat (experiments 47 and 48). Five of the seven were from mating 47, and the remaining two from mating 48.

Three of the albinoes used (experiments 60, 62, and 63) were also derived from the same albino parents, which were obtained from ordinary dealers. In experiment 61 the albino parent was also obtained from the same source, but from a different parentage. Their zygotic composition may therefore be represented by cgBsP. In experiments 64, 65, and 66 the albinoes used were extracted ones. In 64 it was extracted from a mating of two "Irish" *b* parents (experiment 50). In experiments 65 and 66 the two albinoes used were brothers, and were extracted from a cross between a Cr 2 × Cr 5 (experiment 38).

I will therefore deal with experiments 60, 61, 62, and 63 together, since all the four albino parents are probably of the same gametic constitution.

The expectation is :—

2 Cr 4 + 1 Cr 5 + 1 Cr 6 *b* = 1 alb. : 1 pigmented.

The actual result of the four combined experiments is :—

$$14\ \mathrm{Cr}\ 4+\underbrace{7\ \mathrm{Cr}\ 5+3\ \mathrm{Cr}\ 6\ b+6\ \text{pigmented eyes.*}}$$

14 alb. : 16 pigmented. = 1 : 1·1.

Result and prediction are thus almost identical with regard to the proportion of albinoes and pigmented individuals; they are so with regard to the nature of the types predicted, and doubtless had the six individuals with pigmented eyes lived, there would have been a near approach to expectation of the Cr 6 *b* type.

Experiments 65 *and* 66.

As already stated, the albino parents in this cross were derived from a Cr 2 × Cr 5 (experiment 38). The Cr 2 was derived from wild grey × Cr 4 (of ordinary dealers) (experiment 20).† The Cr 5 was derived from Cr 6 *b* × Cr 4 (of ordinary dealers) (experiment 62). The Cr 6 *b* from black variety grey × Cr 4 (of ordinary dealers) (experiment 48). (See Descendants' Table, Charts II and III.)

From an ancestry of this nature the albino parent used in experiments 65 and 66 may have one of four zygotic compositions, *i.e.*, cgBsP, cgBSsP, cGgBsP, cGgBSsP. The "Irish" *b* parents will be CcgBSsP.

If the albinoes are of the nature indicated by the last two symbols, and are carrying the grey determiner, then Cr 2 individuals are expected in the offspring. They are absent. Albinoes and the Cr 5 type are also expected in any case, but the Cr 4 is absent in experiment 65 and the Cr 5 in experiment 66. The numbers, however, are small, and it has been shown that expected types do not always appear in the first litter. The results of these two experiments do not coincide in their proportions with prediction, and they are being repeated on a larger scale.

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* These died before coat colour had developed.

† This experiment will be described in a later paper.

Experiment 64.

In this experiment the albino was extracted from a cross of Cr 6 *b* × Cr 6 *b* (experiment 50). Reference to the table of gametic combinations on p. 103 will show that an albino thus extracted may be either cgBsP, cgBSP, or cgBSsP.

If it be cgBSP, then a Cr 6 *a* is expected in its offspring when crossed with a Cr 6 *b*; if it be cgBSsP, then a Cr 5 is additionally expected.

Assuming it to be cgBSsP, the full expectation is

$$4 \text{ Cr } 4+1 \text{ Cr } 5+1 \text{ Cr } 6\, a+2 \text{ Cr } 6\, b.$$

The actual result of this mating in two litters is 3 Cr 5 + 2 Cr 6 *a* + 8 Cr 6 *b*. The expected albinoes are therefore missing, and there is a preponderance of the Cr 6 *b* type. But since the Cr 5 and Cr 6 *a* types were both absent in the first litter, it is possible that the expected Cr 4 types would have appeared had the parents lived to produce a third litter.

The appearance, however, of the Cr 6 *a* type among the offspring can only occur if the parental albino is carrying the self-pattern determiner, and the presence of the Cr 5 type can only occur if it carries an allelomorph, *i.e.*, s, which, being recessive to it, will therefore allow P to be manifested. The presence of Cr 6 *a* together with that of the Cr 5 type, must therefore be regarded as initial evidence that this type of albino has been actually produced. And, since it is predicted (see table of gametic combinations, p. 103) that some of the albinoes produced in a cross of Cr 6 *b* × Cr 6 *b* will be of the two types cgBSP and cgBSsP, it thus far constitutes a fulfilment of the prediction of the existence of this latter type in contrast to the former.

(4) *"Irish" b × Grey with White = Cr* 6 *b* × *Cr* 2.

One cross only (experiment 66*a*) of this nature has been made, but the result is of exceptional interest, and affords a cogent test of the doctrine of gametic purity and of dominance.

To understand the case, it is necessary to consider the origin of the Cr 2 member of the pair. Its parents were a black variety of the wild grey rat (experiment 48) × with an albino purchased of ordinary dealers. In this mating there were seven young, six being of the expected "Irish" *b* type and one exceptional one, *i.e.*, a Cr 2. In my other crosses of this nature there were 13 young, all of the "Irish" *b* type.

To explain the production of this Cr 2, it is necessary to assume that the albino rat which was mated to the black variety of grey was carrying the grey determiner.* For reasons which will appear later, I will further assume that the albino also carried the black and piebald pattern determiners. Its composition will then be represented by cGgBsP. Such an albino, mated with a homozygote black, will produce, according to the subjoined Table II, equal numbers of "Irish" *b* and Cr 2.

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* The same result would be obtained by supposing that the black variety of wild grey was carrying the grey chromogen. But since grey is dominant to black this cannot be the case.

Table II.—Predicted Offspring in a Cross of Cr 7 (extracted) × Cr 4 (extracted). The albino is carrying the grey determiner and may be either cGgBsP or cGgBSsP.

Gametes formed by self black.		Gametes formed by albino.		Zygotic composition of the offspring.		Visible zygotic characters of the offspring.
CgBSP	×	cgBsP	=	CcgBSsP	=	Cr 6 *b*
CgBSP	×	cGBsP	=	CcGgBSsP	=	Cr 2
CgBSP	×	cGBSP	=	CcGgBSP	=	Cr 2
CgBSP	×	cgBSP	=	CcgBSP	=	Cr 6 *a*

Total Offspring.

If albino = cGgBsP = 1 Cr 2 + 1 Cr 6 *b*.
" = cGgBSsP = 2 Cr 2 + 1 Cr 6 *b* + 1 Cr 6 *a*.

If the assumption that the albino parent is carrying the grey determiner be correct, then the exceptional Cr 2 member among the offspring, in experiment 48, may be assumed to be of the zygotic composition CcGgBSsP.

If we ascertain the possible gametic combinations when such a Cr 2 individual is crossed with an "Irish" *b* of CcgBSsP composition, it will be such that the F 1 offspring predicted will be as follows:—

8 Cr 4 + 1 Cr 1 + 8 Cr 2 + 3 Cr 3 + 3 Cr 5 + 2 Cr 6 *a* + 6 Cr 6 *b* + 1 Cr 7
= 1 Cr 4 : 3 pigmented.

Now, the special feature in this progeny is the presence of the black forms, especially the homozygote Cr 7. Grey being dominant to black, black individuals could not appear in the F 1 generation of a cross between grey (Cr 2) and black ("Irish" *b*), unless the grey member carried black recessive.

Therefore, if black members of any coat pattern actually appeared in the F 1 generation of this cross of "Irish" *b* × Cr 2, it must be accepted as evidence that the Cr 2 carried black recessive. And, since on no other hypothesis, as far as I can see, could these black members appear—bearing in mind the unquestioned dominance of grey—unless gametic purity or segregation of unit characters really occurs, it must also be accepted as a cogent proof of the truth of this doctrine.

The actual offspring (experiment 66*a*) consisted of:—

1 Cr 4+1 Cr 1+2 Cr 2+1 Cr 5+1 Cr 7.

Thus, two types of black individuals have appeared, *i.e.*, Cr 5 and Cr 7. The predicted "Irish" form, together with the grey Cr 3 type, is absent. But if we bear in mind that, at the lowest limit, eight individuals at least must be produced in order to represent all types expected, and that actually only a total of six individuals were produced, it is obvious that two types at least must be absent. Unfortunately, though I kept these two rats mated for a year and a-half after this first litter, they never gave rise to a second one, and are now dead.

If the hypothesis above made be correct, then the Cr 7 member of the offspring should be homozygote. It was tested by mating with three albinoes (experiments 43, 44, 45) in succession, the last of which carried the grey determiner. With the first two albinoes, it had a single litter in each case, of eight individuals, making a total of 16, all of the "Irish" *b* type. No albinoes appeared. The "Irish" *b* individuals of this offspring were crossed *inter se* (experiments 51, 52, 53), and gave a progeny wholly confirmative of the homozygote nature of the Cr 7. When crossed (experiment 45) with the third albino (carrying the grey determiner), it gave a wholly pigmented offspring, five in number (*infra*, p. 114).

Thus, in a total offspring of 21, from three albinoes mated with it, not a single albino appeared. There can, then, be no doubt as to the homozygote nature of the Cr 7.

Testing the assumption further by following some other of the offspring of the F 1 generation of this "Irish" *b* × Cr 2, to the F 3 generation, we may consider the behaviour of two of the F 2 Cr 2, which I mated with albinoes (Pedigree Chart I, experiments 73 and 74).

If the assumption be correct that the Cr 2 parent (experiment 66*a*) carried B and P recessive, then the Cr 2 members of its offspring should be of several zygotic compositions, *i.e.*, CcGgBSP, CGgBSsP, and CcGgBSsP.

If the F 2 Cr 2 of CGgBSsP composition be mated with an albino of reputed cgBsP composition, two particular features are expected in the F 3 generation. There should be no albinoes, and black forms should appear. The expected offspring is:—

1 Cr 2+1 Cr 3+1 Cr 5+1 Cr 6 *b*.

In experiment 73, an F 2 Cr 2 was mated with an albino derived from albino grandparents obtained from an ordinary dealer, and presumably, therefore, of cgBsP composition. The actual result is:—

2 Cr 2+4 Cr 3+5 Cr 5.

The two special features predicted are therefore present, *i.e.*, absence of albinoes and presence of black forms. We may therefore regard this experiment as being initial evidence that a Cr 2 of the predicted composition CGgBSsP was produced. Of course, the experiment must be repeated, and larger numbers reared before it can be accepted as proved.

But it is also predicted that an F 2 Cr 2 of composition CcGgBSsP will be formed. The offspring of such a member when it is crossed with an albino of cgBsP composition will differ from the one just considered only in the presence of albinoes. In experiment 74, the second F 2 Cr 2 was crossed with an albino which had been extracted from a Cr 2, and may therefore have been carrying the grey determiner, as well as the black one.

If the albino carried the black determiner only, the expected offspring is—

4 Cr 4+1 Cr 2+1 Cr 3+1 Cr 5+1 Cr 6 *b*.

If it carried the grey determiner additionally, it will be :—

8 Cr 4+3 Cr 2+3 Cr 3+1 Cr 5+1 Cr 6 *b*.

The actual result is :—

15 Cr 4+1 Cr 2+1 Cr 3+4 pigmented eyes.*

In the presence of albinoes, it satisfies one of the two special features of the prediction, though the absence of the black forms is disappointing. It is probable that the four individuals with pigmented eyes, which died quite early, included some black types. The presence of the albinoes is evidence that the Cr 2 contained albinism recessive. And such a type was predicted.

Thus on the basis of the hypothesis of the nature of the Cr 2 of the F 1 generation (experiment 48), it is predicted that the Cr 7 type of the F 2 generation (experiment 66*a*) shall be homozygous. And prediction in this case has been completely proved to be fulfilled. It is also predicted that among the Cr 2 members of the F 2 generation (experiment 66*a*), three types shall be produced (*supra*, p. 110), and of these, two have been shown to very probably exist, by the nature of the F 3 generation (experiments 73 and 74).

(5) *Zygotic "pattern" Albinoes.*

There remains one feature of importance to mention. It is one almost new to science, and, as far as I know, has been observed only by Haacke (19), and then only in the case of a single individual.

In experiment 74, just considered, where a particular Cr 2 was × Cr 4, there occurs in the F 3 offspring 15 albinoes. Of these, six were born in the second litter. Now, it is predicted that albinoes of such parents may include six zygotic types, *i.e.*, cgBsP, cgBSsP, cGBsP, cGgBsP, cGBSsP, and cGgBSsP.

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* These died before the coat colour or pattern had developed.

With regard to the positive pattern determinants which they carry, we may regard them as of two types, *i.e.*, those carrying only P, and those carrying both P and S. It should be noted that in pigmented individuals S is known to be dominant to P.

Hitherto, we have had to rely upon breeding tests in order to ascertain what pattern determinants are carried by albinoes. But of the six albinoes in the second litter of this cross, one died early, while three of the remaining five had quite distinctly the pattern of the piebald rat. One had also the pattern of a Cr 2 or Cr 6 *b* (these two patterns are the same, only the colour differs), and the remaining one had that of a self-coloured individual.

I do not purpose going into details here as I shall publish a full account in another paper. It will be sufficient to say that the pattern is rendered visible by some difference in the texture and closeness of arrangement of the hairs, so that where in pigmented individuals there is colour, in albinoes there is pinkness, due to the fact that the underlying skin shows through; and where in pigmented individuals there is absence of pigment, in albinoes there is an opaque whiteness, the skin not showing at all. Thus, in the "self-pattern" albino mentioned above, the whole surface of the body exhibited a pinkness.

The visibility of the "pattern" early disappears, for I first noticed it in early August when the young were a month old, and it had disappeared in early October. In more recent cases I have noticed that it disappears when the individuals are about two to two and a-half months old.

It is thus in the highest degree probable that some of the piebald marked albinoes were of the cgBsP, cGBsP, or of the cGgBsP type, that the Cr 2 or Cr 6 *b* pattern was of the cgBSsP or cGgBSsP type, while the self-pattern one may have been of the cGBSsP type.*

If this conclusion is valid, then the prediction which forecasts that a homozygote black shall be formed, that certain Cr 2 types shall also be formed, and which also predicts that certain pattern bearing albinoes will be produced, has in all these cases been fulfilled.

Haacke's single case (18) was evidently a piebald-bearing albino. The other types are here described for the first time. Lock has described a somewhat analogous case for the "maple" markings in peas (21).

(6) *Self-black* × *Albino* = *Cr* 7 × *Cr* 4.

(a) *Black Variety of Wild Grey* × *Cr* 4.—Two crosses of this kind were made (experiments 47 and 48).

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* While this paper was passing through the press, this conclusion has been tested by mating a "self-pattern" albino with a Cr 5. There were six young, all Cr 6 *b*. Since self is dominant to piebald, the Cr 5 could not have carried the self-pattern determiner. This result is therefore conclusive proof that the albino did so.

In both matings, the albino parent was obtained from an ordinary dealer; that in experiment 47 from one dealer, and that in experiment 48 from another. It is important to note this fact.

In experiment 47 there were 13 young in one litter, all of the "Irish" *b* type.

In experiment 48, there were seven young, all at first of the "Irish" *b* type, but one of these changed colour and became a Cr 2 in a few weeks after birth. This change of colour has several times occurred in my experiments and will form the subject of a separate paper.

The further history of this Cr 2 member has been already considered, p. 108 and experiment 66*a*.

From the "Irish" *b* individuals, the extracted self-black types to be considered in the next cross were derived, as well as directly or indirectly, all the individuals of the "Irish" *b* crosses described in this paper. The details of the behaviour of these two types will, therefore, be found under their appropriate headings.

(b) *Extracted Self-black* × *Albino.*—Four matings of this kind were made (experiments 43, 44, 45, 46). Although there were four matings, only two black individuals were used, since the same individual ((1) 52/53) was crossed with three different albinoes. In experiments 43 and 44, this self-black individual was crossed with an albino which was derived from albino parents obtained from ordinary dealers. The zygotic composition of the albino may be, therefore, represented as cgBsP. In each of these two matings there were eight young all "Irish" *b*.

Six of these from the second mating (experiment 44) were crossed *inter se* (experiments 51, 52, and 53). There is expected (*supra*, p. 103), on the assumption that their self-black parent is homozygote, albinoes and pigmented individuals in the proportion of 1 : 3 respectively. Actually it is as near as it can possibly be, *i.e.*, 4 : 11.

The detailed expectation (Table I, p. 103), is:—

$$4 \text{ Cr } 4+3 \text{ Cr } 5+2 \text{ Cr } 6\, a+6 \text{ Cr } 6\, b+1 \text{ Cr } 7.$$

There are produced:—

$$4 \text{ Cr } 4+3 \text{ Cr } 5+0 \text{ Cr } 6\, a+6 \text{ Cr } 6\, b+2 \text{ Cr } 7.$$

There is thus one Cr 7 too many and the expected "Irish" *a* is missing. But since some "Irish" *a* individuals have only a few white hairs on the chest, it is possible that they may be mistaken for Cr 7 until they are gametically tested. The result, therefore, may be regarded as confirming prediction in a very exact manner.

These "Irish" members, combined with the similar offspring of other crosses, have been already considered (*supra*, p. 102).

In experiment 45, the same self-black rat was crossed with an albino which had been extracted from a cross of Cr 2 × Cr 4 (experiment 71). The Cr 2 was derived from a cross of a wild grey × albino.

In experiment 46, a very similar cross was made, but the self-black member was extracted from a cross between two "Irish" *b* individuals (experiment 50). The albino used in this mating (experiment 46) was sister to that used in experiment 45. We may, therefore, consider experiments 45 and 46 together. The offspring will depend upon the nature of the Cr 4 parent. It will be assumed that the self-black parent in each case is homozygote, and in fact that used in experiment 45 has been proved to be so, by the previous results of 43 and 44.

Albinoes extracted from a cross of Cr 2 × Cr 4, may be expected to include individuals of the following zygotic characters: cgBsP, cgBSsP, cGgBsP, and cGgBSsP (*supra*, p. 111). If the albino is cGgBsP, and it is crossed with a homozygote Cr 7, the prediction (Table II, p. 109) is:—

1 Cr 2 + 1 Cr 6 *b*.

If the albino is cGgBSsP, the prediction is:—

2 Cr 2 + 1 Cr 6 *b* + 1 Cr 6 *a*.

In the two experiments (45 and 46) which we are considering, the total offspring is:—

11 Cr 2 + 8 Cr 6 *b* + 1 Cr 6 *a*.

In experiment 46 no Cr 6 *a* type appeared, and it therefore suggests that the albino parent is cGgBsP, and probably differing from that in experiment 45, in the absence of S.

If, therefore, we take experiment 45 alone the result is:—

2 Cr 2 + 2 Cr 6 *b* + 1 Cr 6 *a*.

This is as near as it is possible to get to expectation with the odd numbers obtained. The presence of grey offspring in both experiments is conclusive proof that the two albinoes carried the grey determiner.

Thus in this case, prediction as to the nature of the Cr 2, of the two extracted Cr 4 individuals, and of the two Cr 7* individuals, is tentatively fulfilled.

The behaviour of the black individual in the manner described, in experiments 43, 44, and 45, shows in a marked manner the influence which the albino parent exerts upon the nature of the offspring. In experiments 43 and 44 it had none but "Irish" *b* members in its offspring, 16 in number, because the albino with which it was mated had come probably from a piebald ancestry, and was carrying nothing but piebald pattern and black determiners. But in experiment 45 it was mated with an albino in which it was probable that the grey determiner was present, because of its extraction from a grey parent. And grey individuals then appeared in its progeny. Grey individuals also appeared in the offspring of a sister albino when it was mated with a homozygote self-black.

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* One of these two (*i.e.*, that of experiment 45) is the same individual as that considered on p. 110.

(7) *Summary of Results.*

The figures within brackets indicate the page where the matter of the summary may be found.

1. That black is dominant to albinism (113). The dominance is not complete, some ventral white being always present. In relation to dominance this is perhaps not of particular significance, since ventral white patches are known to occur in both grey and black wild rats. This conclusion is confirmatory of that deduced from Crampe's results, and arrived at in Doncaster's experiments.

It is theoretically conceivable that the incompleteness of the dominance is due to the influence of the factor s (= absence of self-pattern) brought in by the albino.

2. Expected types do not all always appear in any one litter of young, and frequently not until the third litter (106).

Doncaster has also shown that there is considerable diversity in the proportions of the indivduals of each type in successive litters, but the examples published by him do not show whether a type present in one litter may be absent in another, as in several of my experiments.

3. Predictions based upon the doctrine of gametic purity and of dominance have not in these experiments (including those not yet published) been falsified by the appearance of unpredicted types. With but few exceptions, the predictions with respect to the kind of types expected are fulfilled, and approximately so with respect to proportions. The exceptions are due to the smallness of the number of offspring in certain cases.

4. The conception (hitherto a matter of deduction) that albinoes are the bearers of hidden factors, has been ocularly demonstrated for the piebald, "Irish" and self patterns (111). In peas a somewhat analogous phenomenon has been seen by Lock.

5. That completely self-black extracted forms are homozygous for black (110, 113).

This conclusion appears not to harmonise with some of Doncaster's results, *i.e.*, experiments 34 and 37, where albinoes appeared in a cross when one of the parents is described as black.

Since the black parent was the same in both experiments, and its parents were unknown, it is not improbable that it was an "Irish" *a*, and not a true black. If we make this assumption and accept the conclusion as to the nature of the Cr 6 *a* given in this paper (Conclusion 7), then the results of Doncaster's experiments 34 and 37 are in accordance with prediction.

6. The "Irish" "*b*" type is a heterozygote. Two zygotic forms have probably been experimentally recognised, *i.e.*, of the composition CcgBSsP and CgBSsP (104—107).

With regard to this conclusion, the first symbolic representation confirms that of Doncaster, while the second is new.

7. That the "Irish" "*a*" type is perhaps of the composition CcgBSP. At present there is no direct evidence of this; but in the possible theoretical gametic combinations (*supra*, pp. 103 and 109) such a composition occurs. The evidence shows that the "Irish" *b* always carries piebald and s, and this being so, the composition CcgBSP can be ascribed to the "Irish" *a* form alone. Some of Doncaster's experiments (15, p. 225, experiments 35 and 63) show that the "Irish" "*a*" does, in some cases, carry albinism recessive.

Hence his own conclusion, that the Cr 7 is only an extreme type of Cr 6 *a* and is not a separate variety, needs for the present to be accepted with hesitation. My work indicates that the Cr 7 is homozygote for black (see 5). The above considerations suggest that the two varieties are distinct. And, taken in connection with Conclusion 6, suggests that both "Irish" *a* and "Irish" *b* are heterozygotes.

With regard to the composition CcgBSP ascribed to the "Irish" *a*, there is to be remembered Doncaster's conclusion, which I endorse, that the "Irish" *a* and the Cr 7 have given no evidence that they carry P. But there is nothing inconsistent between this conclusion and the symbolic representation of this form, because S and P are not allelomorphic to each other, and, therefore, though P may be present, it cannot be manifested so long as S is also present in the same gamete or zygote. Even if the "Irish" *a* contains P, and it is crossed with an albino carrying P, no piebald forms will be expected in the F 1 generation, because it (the "Irish" *a*) also carries S. Neither are they expected if "Irish" *a* × "Irish" *a*.

Piebald forms cannot appear until s is introduced into the "Irish" gamete or zygote. The evidence indicates that when this is done the "Irish" *b* zygotic form is produced. It seems impossible, therefore, to obtain direct experimental evidence that the "Irish" *a* carries P.

8. That of the various theoretically possible types of albinoes, the following four have in these experiments been shown to exist, *i.e.*, cgBsP (102, 106, 110—111, and 112—113), cGgBsP (109, 112, 114), cgBSsP (108, 112), and cGgBSsP (112, 114). To a lesser extent, the evidence indicates that two other types, *i.e.*, cGBsP and cGBSsP, also exist (112). That is, of the four types experimentally recognised, so far, all carry both the black and piebald factors; one, and perhaps three, in addition, carry the grey factor, another the self-pattern factor, and one, or perhaps two, carry both the grey and self-pattern factors. The type cgBSP has not yet been demonstrated to exist. Of these four or six types, Doncaster has shown the existence of the two first: the others are new for rats. Hurst has shown the same thing for rabbits, *i.e.*, the existence of albinoes carrying B, and of those carrying BG. They also carry in some individuals the self character, in others the Dutch character, and in others both. The same thing has been shown for mice by Cuênot and Allen. There is thus a similarity in the carrying powers of albino rats, mice, and rabbits. In rats and rabbits it is to be noted no albino has yet been obtained which carries G alone, *i.e.*, without B.

9. That self-grey types (Cr 2) may carry both black and piebald recessive. The following two forms have been experimentally recognised, *i.e.*, CGgBSsP (111) and CcGgBSsP (111). One thus carries albinism recessive in addition to the black and piebald determiners, and the other does not. Clearly they correspond to the two races of "Irish" *b* revealed in these experiments.

From some of Doncaster's experiments it can be seen that the Cr 2 type carries B and P recessive, but he made no attempt to ascertain whether different zygotic varieties of this type existed. The discrimination of these two zygotic varieties is therefore new.

10. That piebald black-white types (Cr 5) may be homozygous for black and piebald, or may carry albinism recessive (105, 106). This conclusion can also be deduced from Crampe's experiments; none of the Cr 5 types in Doncaster's experiments appear to have been homozygous, and all carried albinism recessive.

11. Externally considered, similar zygotes may have a different gametic constitution (see above, 6, 8, 9, and 10). In experiments not yet published, it can be shown that self-grey forms not distinguishable in external features from those in 9, do not carry g, and therefore B, though present as a recessive character, cannot appear. Zygotic characters alone are not, therefore, a safe basis for prediction.

This confirms and extends the same conclusion which has been arrived at in all experiments which give Mendelian results.

12. With regard to the number of allelomorphs for colour and pattern, the

evidence shows that there are two pairs for each, *i.e.*, presence and absence of greyness, presence and absence of blackness, presence and absence of selfness, and presence and absence of piebaldness.

13. Doncaster divided Crampe's "Irish" type into *a* and *b* sub-types (100). In this paper I propose a stricter definition of the "*b*" sub-type.

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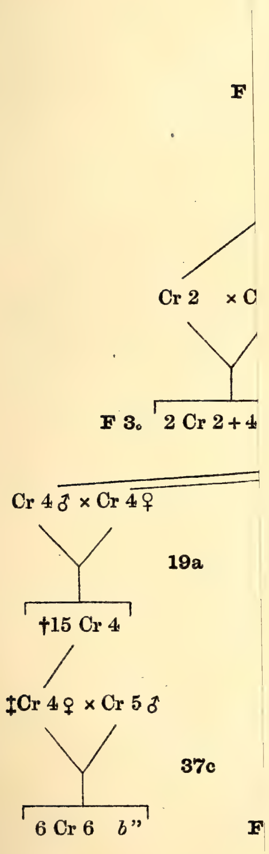
F
Cr 2 × C
F 3₀
2 Cr 2 + 4
Cr 4 ♂ × Cr 4 ♀
19a
†15 Cr 4
‡Cr 4 ♀ × Cr 5 ♂
37c
6 Cr 6 b"
F

NOTE.—In those cases
Pedigree Charts II or II
Ancestry Table (pp. 119—1

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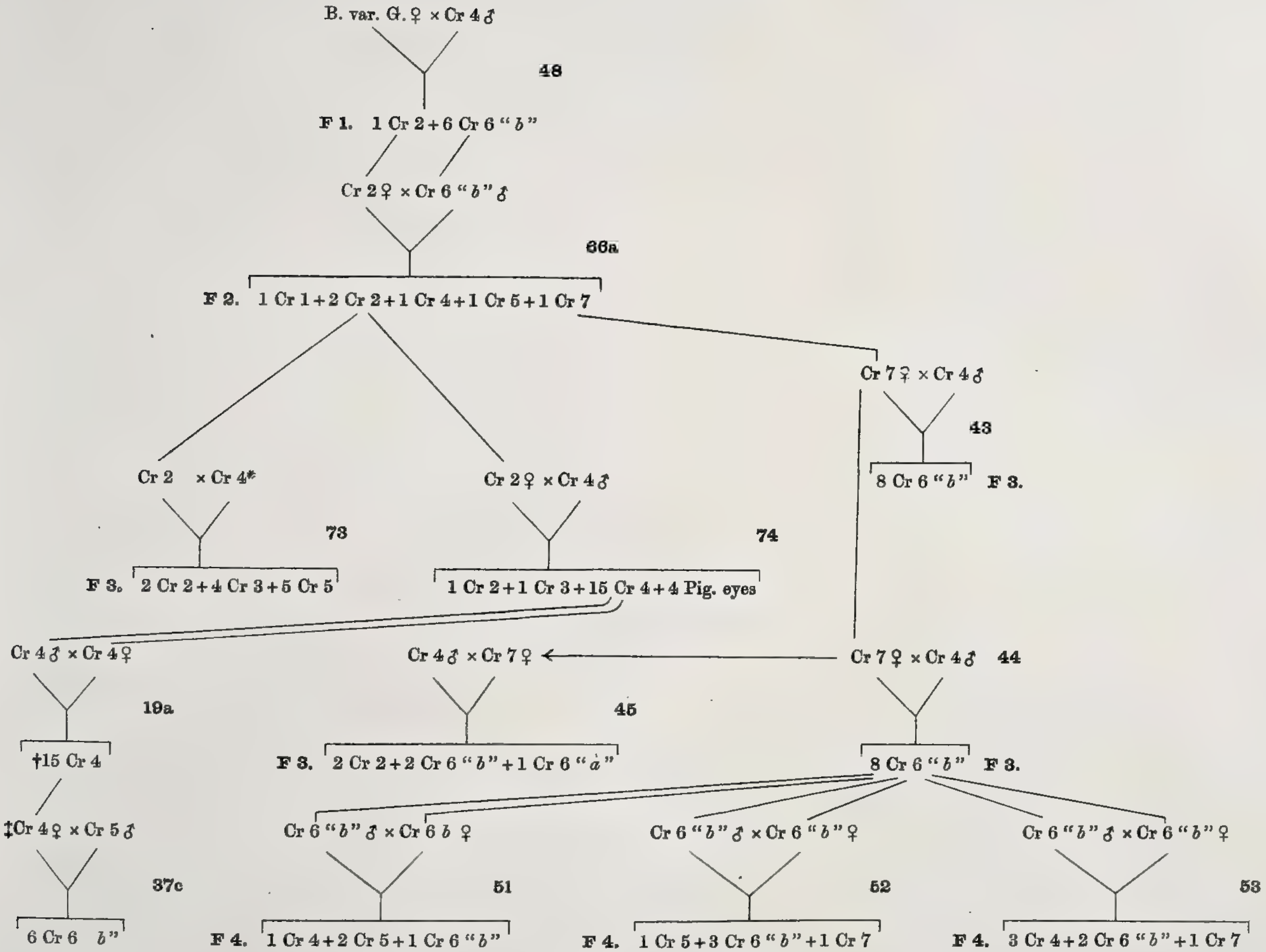
* This
† Seve
‡ This

Pedigree Chart I.

The Descendants of No. 50 × 51.


B. var. G. ♀ × Cr 4 ♂
48
F 1. 1 Cr 2+6 Cr 6 “b”
Cr 2 ♀ × Cr 6 “b” ♂
66a
F 2. 1 Cr 1+2 Cr 2+1 Cr 4+1 Cr 5+1 Cr 7
Cr 7 ♀ × Cr 4 ♂
43
8 Cr 6 “b”
F 3.
Cr 2 × Cr 4*
73
F 3. 2 Cr 2+4 Cr 3+5 Cr 5
Cr 2 ♀ × Cr 4 ♂
74
1 Cr 2+1 Cr 3+15 Cr 4+4 Pig. eyes
Cr 4 ♂ × Cr 4 ♀
19a
†15 Cr 4
‡Cr 4 ♀ × Cr 5 ♂
37c
6 Cr 6 “b”
Cr 4 ♂ × Cr 7 ♀
45
F 3. 2 Cr 2+2 Cr 6 “b”+1 Cr 6 “a”
Cr 7 ♀ × Cr 4 ♂
44
8 Cr 6 “b”
F 3.
Cr 6 “b” ♂ × Cr 6 b ♀
51
F 4. 1 Cr 4+2 Cr 5+1 Cr 6 “b”
Cr 6 “b” ♂ × Cr 6 “b” ♀
52
F 4. 1 Cr 5+3 Cr 6 “b”+1 Cr 7
Cr 6 “b” ♂ × Cr 6 “b” ♀
53
F 4. 3 Cr 4+2 Cr 6 “b”+1 Cr 7

Note.—In those cases where the ancestry of only one of the parents can be traced on this diagram that of the other parent may be found on Pedigree Charts II or III, under the experiment bearing the same number, or it may be found in later papers. It can also be traced in the Ancestry Table (pp. 119—121) of this or later papers.

---

* This albino was obtained from an ordinary dealer.

† Seven of these exhibited the zygotic “ghost” of the self-pattern. The remaining eight died soon after birth.

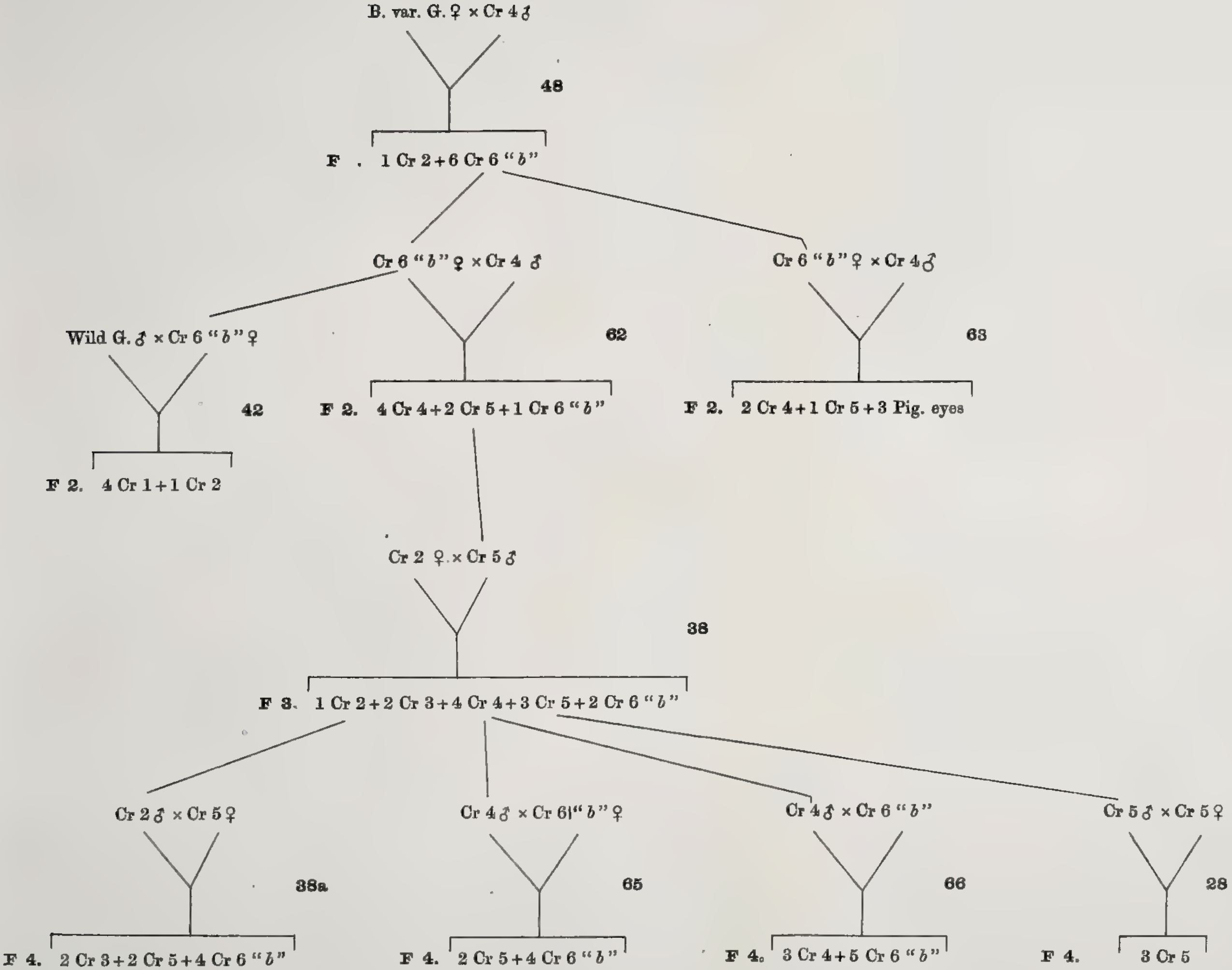
‡ This was one of the seven described in note †.

Wild G. ♂ × Cr 6
F 2. 4 Cr 1 + 1 Cr
Cr 2 ♂ × Cr
F 4. 2 Cr 3 + 2 Cr 5 + 4

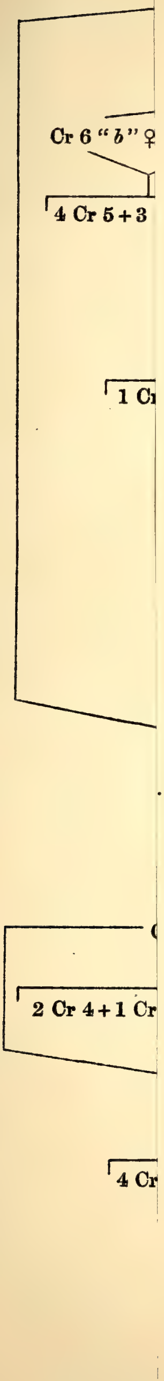
NOTE.—In those cas
Charts I or III, under
Table (pp. 119—121) of

Pedigree Chart II.

The Descendants of No. 50 × 51.


B. var. G. ♀ × Cr 4 ♂
48
F. 1 Cr 2+6 Cr 6 "b"
Cr 6 "b" ♀ × Cr 4 ♂
Cr 6 "b" ♀ × Cr 4 ♂
Wild G. ♂ × Cr 6 "b" ♀
62
63
42
F 2. 4 Cr 4+2 Cr 5+1 Cr 6 "b"
F 2. 2 Cr 4+1 Cr 5+3 Pig. eyes
F 2. 4 Cr 1+1 Cr 2
Cr 2 ♀ × Cr 5 ♂
38
F 3. 1 Cr 2+2 Cr 3+4 Cr 4+3 Cr 5+2 Cr 6 "b"
Cr 2 ♂ × Cr 5 ♀
Cr 4 ♂ × Cr 6 "b" ♀
Cr 4 ♂ × Cr 6 "b"
Cr 5 ♂ × Cr 5 ♀
38a
65
66
28
F 4. 2 Cr 3+2 Cr 5+4 Cr 6 "b"
F 4. 2 Cr 5+4 Cr 6 "b"
F 4. 3 Cr 4+5 Cr 6 "b"
F 4. 3 Cr 5

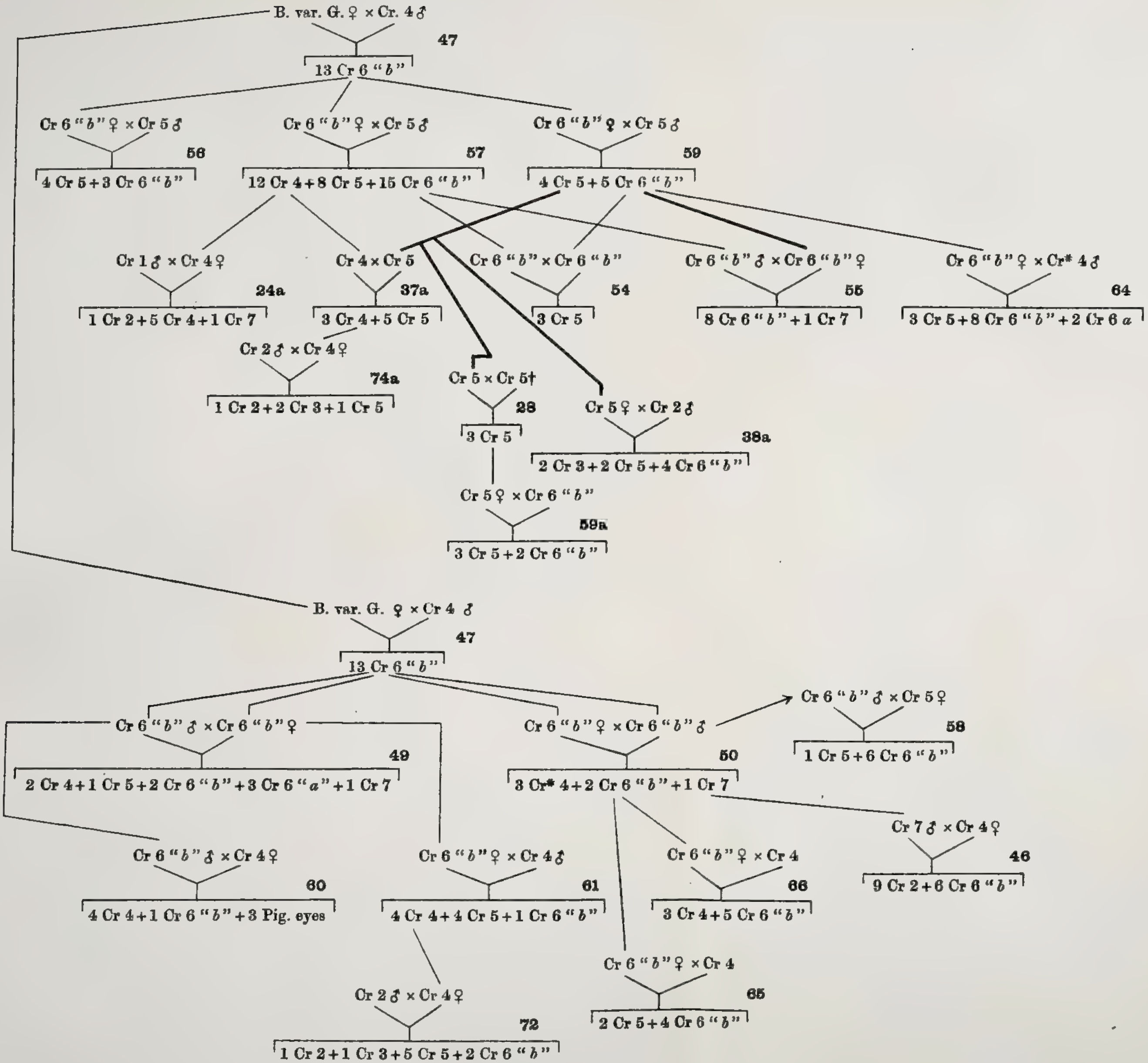
Note.—In those cases where the ancestry of only one of the parents can be traced on this chart, that of the other parent may be found on Charts I or III, under the experiment bearing the same number, or it may be found in later papers. It can also be traced in the Ancestry Table (pp. 119—121) of this or later papers.



Cr 6 "b" ♀
4 Cr 5+3
1 Cr
C
2 Cr 4+1 Cr
4 Cr

PEDIGREE CHART III.

THE DESCENDANTS OF NO. 750 × 152.


B. var. G. ♀ × Cr. 4 ♂
47
13 Cr 6 “b”
Cr 6 “b” ♀ × Cr 5 ♂
56
4 Cr 5 + 3 Cr 6 “b”
Cr 6 “b” ♀ × Cr 5 ♂
57
12 Cr 4 + 8 Cr 5 + 15 Cr 6 “b”
Cr 6 “b” ♀ × Cr 5 ♂
59
4 Cr 5 + 5 Cr 6 “b”
Cr 1 ♂ × Cr 4 ♀
24a
1 Cr 2 + 5 Cr 4 + 1 Cr 7
Cr 4 × Cr 5
37a
3 Cr 4 + 5 Cr 5
Cr 6 “b” × Cr 6 “b”
54
3 Cr 5
Cr 6 “b” ♂ × Cr 6 “b” ♀
55
8 Cr 6 “b” + 1 Cr 7
Cr 6 “b” ♀ × Cr* 4 ♂
64
3 Cr 5 + 8 Cr 6 “b” + 2 Cr 6 a
Cr 2 ♂ × Cr 4 ♀
74a
1 Cr 2 + 2 Cr 3 + 1 Cr 5
Cr 5 × Cr 5†
28
3 Cr 5
Cr 5 ♀ × Cr 2 ♂
38a
2 Cr 3 + 2 Cr 5 + 4 Cr 6 “b”
Cr 5 ♀ × Cr 6 “b”
59a
3 Cr 5 + 2 Cr 6 “b”
B. var. G. ♀ × Cr 4 ♂
47
13 Cr 6 “b”
Cr 6 “b” ♂ × Cr 6 “b” ♀
49
2 Cr 4 + 1 Cr 5 + 2 Cr 6 “b” + 3 Cr 6 “a” + 1 Cr 7
Cr 6 “b” ♀ × Cr 6 “b” ♂
50
3 Cr* 4 + 2 Cr 6 “b” + 1 Cr 7
Cr 6 “b” ♂ × Cr 5 ♀
58
1 Cr 5 + 6 Cr 6 “b”
Cr 6 “b” ♂ × Cr 4 ♀
60
4 Cr 4 + 1 Cr 6 “b” + 3 Pig. eyes
Cr 6 “b” ♀ × Cr 4 ♂
61
4 Cr 4 + 4 Cr 5 + 1 Cr 6 “b”
Cr 6 “b” ♀ × Cr 4
66
3 Cr 4 + 5 Cr 6 “b”
Cr 7 ♂ × Cr 4 ♀
46
9 Cr 2 + 6 Cr 6 “b”
Cr 6 “b” ♀ × Cr 4
65
2 Cr 5 + 4 Cr 6 “b”
Cr 2 ♂ × Cr 4 ♀
72
1 Cr 2 + 1 Cr 3 + 5 Cr 5 + 2 Cr 6 “b”

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* The albino marked thus in Experiment **64** is from the offspring in Experiment **50**.

† The ancestry of this Cr 5 will be found in Pedigree 1000 × 263, Experiment **28**, published in a later paper.

ANCESTRY AND OFFSPRING TABLES.

Explanations.

The "Offspring Columns" include Crampe's seven types, *i.e.*—

Cr 1 = grey with no white.
Cr 2 = grey with ventral white patches and white carpal and metatarsal bands.
Cr 3 = grey-white piebald.
Cr 4 = albino.
Cr 5 = black-white piebald.
*Cr 6 *b* = "Irish" *b*; black with ventral white patterns or patches *and white carpal and metatarsal bands.*
*Cr 6 *a* = "Irish" *a*; black with little ventral white and *no* carpal or metatarsal white bands.
Cr 7 = self black.

* The division of the "Irish" type Cr 6 into "*a*" and "*b*" is due to Doncaster, while I am responsible for the definitions of the two sub-classes as here given.

The number of the parental pairing is given in the column headed Expt. The number of the ancestral pairings will be found in their respective columns.

Experiments from 1 to 41 and from 67 onwards will be given in later papers. A few of these are to be found on the Descendants' Tables (Pedigree Charts I to III).

Expt.	G-G-G-P.	G-G-P.	G-parents.	Parents.	Offspring.							
					Cr 1.	Cr 2.	Cr 3.	Cr 4.	Cr 5.	Cr 6.		Cr 7.
										“*b*.”	“*a*.”	
	See Expt.—	See Expt.—	See Expt.—									
42	—	—	—	Wild grey ♂ 850	4	1	—	—	—	—	—	—
	—	—	48	Cr 6 “*b*” 55								
43	—	48	66A	Cr 7 ♀ (1)52/53	—	—	—	—	—	8	—	—
	—	—	5	Cr 4 ♂ (1)400/401								
44	—	48	66A	Cr 7 ♀ (1)52/53	—	—	—	—	—	8	—	—
	—	—	5	Cr 4 ♂ (2)400/401								
45	—	48	66A	Cr 7 ♀ (1)52/53	—	2	—	—	—	2	1	—
	—	23 and 5	71	Cr 4 ♂ (2)(2)450/6/408								
46	—	47	50	Cr 7 ♂ (6)(3)750/152/(8)750/152	—	9	—	—	—	6	—	—
	—	23 and 5	71	Cr 4 ♀ (6)(2)450/6/408								
47	—	—	—	Blk. var., wild grey (Cr 7) ♀ 750	—	—	—	—	—	13	—	—
	—	—	3	Cr 4 ♂ 152								
48	—	—	—	Blk. var., wild grey (Cr 7) ♀ 50	—	1	—	—	—	6	—	—
	—	—	—	Cr 4 ♂ 51								
49	—	3	47	Cr 6 “*b*” ♂ (1)750/152	—	—	—	2	1	2	3	1
	—	3	47	Cr 6 “*b*” ♀ (4)750/152								
50	—	3	47	Cr 6 “*b*” ♂ (8)750/152	—	—	—	3	—	2	—	1
	—	3	47	Cr 6 “*b*” ♀ (3)750/152								
51	48	66A and 5	44	Cr 6 “*b*” ♂ (1)52/53/(2)400/401	—	—	—	1	2	1	—	—
	48	66A and 5	44	Cr 6 “*b*” ♀ (2)52/53/(2)400/401								
52	48	66A and 5	44	Cr 6 “*b*” ♂ (3)52/53/(2)400/401	—	—	—	—	1	3	—	1
	48	66A and 5	44	Cr 6 “*b*” ♀ (4)52/53/(2)400/401								
53	48	66A and 5	44	Cr 6 “*b*” ♂ (5)52/53/(2)400/401	—	—	—	3	—	2	—	1
	48	66A and 5	44	Cr 6 “*b*” ♀ (6)52/53/(2)400/401								
54	3	47 and 25	59	Cr 6 “*b*” ♂ (1)(9)750/152/252	—	—	—	—	3	—	—	—
	3	47 and 25	57	Cr 6 “*b*” ♀ (1)(7)750/152/266								
55	3	47 and 25	57	Cr 6 “*b*” ♂ (3)(7)750/152/266	—	—	—	—	—	8	—	1
	3	47 and 25	59	Cr 6 “*b*” ♀ (4)(9)750/152/252								

56	—	3	47	Cr 6 "*b*" ♀ (5)750/152	—	—	—	—	4	3	—	—
	—	25	26	Cr 5 ♂ (1)252/253								
57	—	—	47	Cr 6 "*b*" ♀ (7)750/152	—	—	—	12	8*	15	—	—
	—	—	25	Cr 5 ♂ 266								
58	—	—	47	Cr 6 "*b*" ♂ (8)750/152	—	—	—	—	1	6	—	—
	—	—	25	Cr 5 ♀ 267								
59	—	—	47	Cr 6 "*b*" ♀ (9)750/152	—	—	—	—	4	5	—	—
	—	—	25	Cr 5 ♂ 252								
59A	—	20	38	Cr 6 "*b*" ♂ (7)(1)1000/263(2)55/(4)153/154	—	—	—	—	3	2†	—	—
	47 and 25	59 and 38	28	Cr 5 ♀ (1)(5)(9)750/152/252/(1)(1)1000/263/(2)55/(4)153/154								
60	—	—	47	Cr 6 "*b*" ♂ (1)750/152	—	—	—	4	—	1‡	—	—
	—	—	3	Cr 4 ♀ 154								
61	—	—	47	Cr 6 "*b*" ♀ (4)750/152	—	—	—	4	4	1	—	—
	—	—	5	Cr 4 ♂ 403								
62	—	—	48	Cr 6 "*b*" ♀ 55	—	—	—	4	2	1	—	—
	—	3	7	Cr 4 ♂ (4)153/154								
63	—	—	48	Cr 6 "*b*" ♀ 54	—	—	—	2	1	—‡	—	—
	—	3	14	Cr 4 ♂ (1)155/156								
64	—	47 and 25	59	Cr 6 "*b*" ♀ (2)(9)750/152/252	—	—	—	—	3	8	2	—
	3	47	50	Cr 4 ♂ (1)(3)750/152/8/750/152								
65	3	47	50	Cr 6 "*b*" ♂ (2)(3)750/152/(8)750/152	—	—	—	—	2	4	—	—
	48 and 3	62	38	Cr 4 ♀ (2)(1)1000/263/(2)55/(4)153/154								
66	3	47	50	Cr 6 "*b*" ♀ (3)(3)750/152/(8)750/152	—	—	—	3	—	5	—	—
	48 and 3	62	38	Cr 4 ♂ (5)(1)1000/263/(2)55/(4)153/154								
66A	—	—	48	Cr 6 "*b*" ♂ 53	1	2	—	1	1	—	—	1
	—	—	48	Cr 2 ♀ 52								

* There were two other individuals with pigmented eyes, but they died before coat colour developed.
† One individual with pigmented eyes died.
‡ There were three other individuals with pigmented eyes, but they died before coat colour had developed.

On the Result of Crossing Round with Wrinkled Peas, with Especial Reference to their Starch-grains.

By A. D. Darbishire, Royal College of Science, London.

(Communicated by J. Bretland Farmer, F.R.S. Received June 20,—Read November 14, 1907.)

One of the characters with which Mendel dealt in his hybridisation experiments with peas was, as is well known, the shape of the ripe seed. Weldon, in his criticism* of Mendel's interpretation of his results, showed that round were not discontinuously distinct from wrinkled peas, but that intermediate shapes connecting these two extremes were not infrequently exhibited. The answer which was made to Weldon's criticism was that the intermediate shapes were due to spurious pitting or dimpling of the seed, and did not represent an intermediate condition of the germ which gave rise to them. And this answer was shown to be correct by the work of Gregory,† who found that the starch-grains of round and wrinkled peas were quite distinct, and that they afforded an infallible test by which the real character of a pea with doubtful shape could be determined.

Our knowledge of this subject has not advanced beyond the stage reached by Gregory in 1903; that is to say, we know no more about the inheritance of wrinkledness and roundness than Mendel did, except that each of these two characters is associated with a particular kind of starch-grain. What is the nature of the starch-grain in the hydrid; and how the characters of the starch-grains segregate, if they do so at all, in subsequent generations, are points on which we are at present ignorant. The observations, which I have to record, form the first instalment of an attempt to fill up this gap in our knowledge.

Part of the cost of these experiments was defrayed by a Government Grant.

The Starch-grains of the Round Pea.

The grains in a round pea such as "Eclipse" are, as shown in fig. 1,‡ single, potato-shaped grains. They will be referred to for the sake of brevity as *p*-grains. Their average length is 0·0322 mm., their average breadth

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* 'Biometrika,' vol. 1, p. 231.

† 'The New Phytologist,' vol. 2, No. 10, p. 226.

‡ Fig. 1 is magnified slightly less than figs. 2 and 3. The actual sizes of the types of grains figured is given in the text. All the grains described have been derived (as far as possible) from the *deeper* layers of the cotyledon as distinguished from the *superficial* ones where the grains are smaller.

0·0213 mm. These measurements are accurate to 2 μ. The length-breadth index (*i.e.*, 100 × breadth-length) is 66·14. The above figures were calculated from a series of measurements of 232 grains.

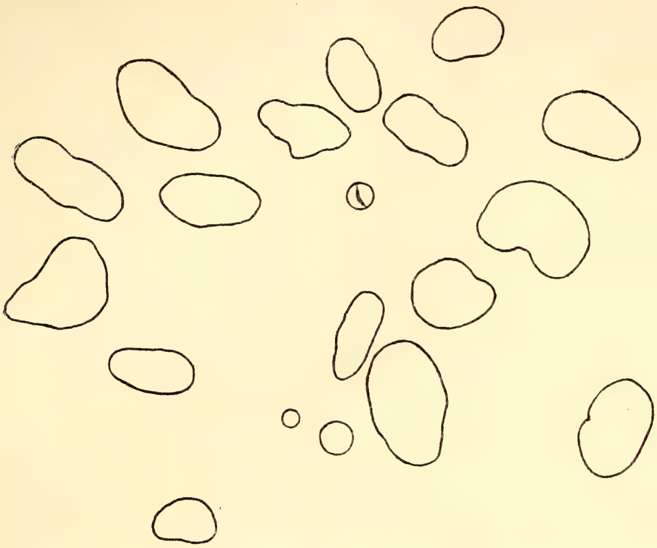

Fig. 1.—Starch-grains of Round Pea (*p*-grains).

Beside the large potato-shaped grains which have just been described there are always found associated with them extremely few very much smaller grains which are *round.* Two of these are shown in fig. 1. These small round grains occasionally exhibit traces of division, as in the case of the third small grain shown on fig. 1. The division does not affect the contour of the grain and I have not found a small grain divided into more than two subdivisions.

The Starch-grains of the Wrinkled Pea.

The starch-grains in a wrinkled pea like "British Queen" are, as shown in fig. 2, immediately distinguishable from those of the round pea, by the fact that they are compound. They will be referred to for the sake of brevity as *c*-grains. Each grain consists of a number of pieces which varies between 2 and 8. The separate pieces are loosely held together by a refringent yellow substance which, from the fact that it does not stain blue with iodine, appears to be of a different nature from the pieces themselves.

These grains are very liable, as Gregory pointed out, to break up into their component pieces. I do not propose to go into the question of the relation between the number of component pieces and the size of a grain. Suffice it to say that the examination of a large number of samples reveals a rough positive correlation between size of grain and number of component pieces. The commonest types of grains are those with 4, 5 or 6 component pieces. Grains with 7 or 8 pieces are rarer; grains with 2 or 3 pieces are intermediate in frequency between those with 4, 5 and 6 on the one hand and 7 and 8 on the other. But whilst grains with 7 or 8 pieces are not much larger than those with 4, 5 or 6, grains with 2 or 3 are always conspicuously smaller than those with 4, 5 or 6.

The average length of the grains is 0·0269 mm., the average breadth 0·0248 mm. The average length-breadth index is 92·19; that is to say, the grains are, as a glance at fig. 2 shows, almost round. The above figures were calculated from measurements of 105 grains.

There are also in wrinkled peas a number of very small single grains which can be distinguished from the pieces, into which the compound grains break up, by the fact that they are circular. They are always smaller than grains consisting of two pieces. Two of them are shown in fig. 2.

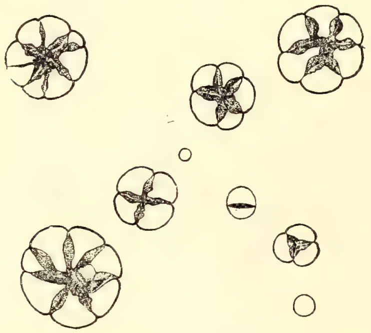

Fig. 2.—Starch-grains of Wrinkled Pea (*c*-grains).

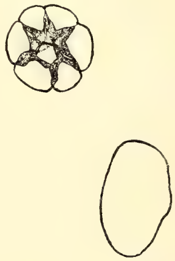

Fig. 3.—Normal *c*-grain of Wrinkled Pea, together with occasional *p*-grain.

Lastly, I have found very rarely indeed, in examples of "British Queen," isolated *p*-grains, as shown in fig. 3. At first I thought these grains had been introduced by using a razor with which I had been cutting round peas. But after taking every precaution to guard against this, by immersing the razor in strong potash before using it, I still found them: so that I cannot but believe that they really occur in wrinkled peas. I have also found them "Telephone," and in extracted wrinkleds.

The Starch-grains of the Hybrid.

For the examination of the characters of the grains of F_1 cotyledons I had, unfortunately, only two seeds left from a number of crosses between "British Queen" and "Eclipse" which Mr. J. T. Wadsworth made for me in the summer of 1905.* All the rest had been sown: the plants bearing the F_3 cotyledons raised from them are now in flower.

The starch-grains in the F_1 cotyledons produced by crossing a round with a wrinkled pea are not like those of the round pea, as might have been expected from the fact that round is dominant over wrinkled, but are intermediate between those of the round and those of the wrinkled pea.

In the first place they are nearly round (see fig. 4), and will on this account and for brevity's sake be referred to as *r*-grains.

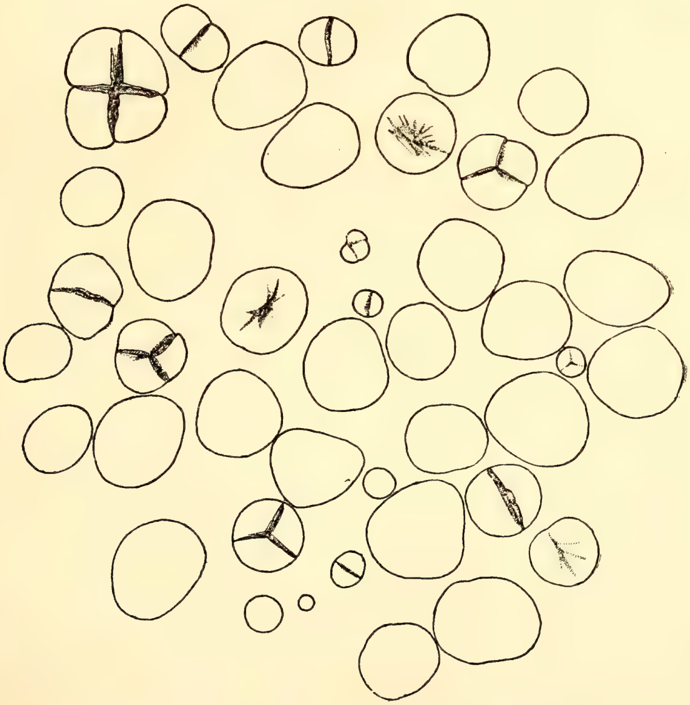

Fig. 4.—Starch-grains of F_1 cotyledons (*r*-grains).

In the second place, whilst the majority of the grains in the F_1 seeds are single the remainder are compound. In a count of 579 grains, 356 were single, 223 were compound. In the third place the degree of compoundness exhibited by the compound grains in F_1 seeds is intermediate between singleness and the degree of compoundness in the grains of wrinkled peas. For whilst in these latter the number of pieces varies between 2 and 8 and the commonest is 6, in the F_1 grain it varies between 2 and 4 and the commonest is 3.

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* For the nature of the starch grains in other crosses between round and wrinkled peas, see pp. 132, 133.

Table I gives the average length, breadth, and length-breadth index of 166 *r*-grains, side by side with the measurements for *p*- and *c*-grains for comparison.

Table I.

	Round.	F_1.	Wrinkled.
	p-grain.	*r*-grain.	*c*-grain.
Average length	0·0322	0·0276	0·0269
Average breadth	0·0213	0·0236	0·0248
Length-breadth index	66·14	85·5	92·19

This table shows that, in the matter of shape, the F_1 grain is intermediate between the *p*- and the *c*-grain, but *nearer the* c-*grain.*

The Starch-grains in a Subsequent Generation.

I have not at present any information as to the nature of the grains in F_2, but I have some data relating to F_5 which throw much light on the mode of segregation in this case.

By a fortunate chance I happened, when I turned my attention to this subject, not to have sown the contents of 48 packets of F_5 peas;* each separate packet of which contained five seeds from a single plant. Thirty-six of the packets contained round seeds; 12 of them contained wrinkled ones. I possessed a record of the number of green and yellow, as well as, what concerns us now, the number of round and wrinkled seeds produced by each of the plants, five of each of whose seeds were preserved in the packets. So that I could find out in the case of the seeds in any given packet whether they were borne on a plant which produced only rounds, or both rounds and wrinkleds, or only wrinkleds. I wish particularly to emphasise the fact that this information was not written on the packets, but was obtainable by looking up, in the records, the plant represented by the number on the packet containing its seeds.

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* The history of these seeds is as follows: In the spring of 1905, Mr. C. C. Hurst kindly gave me the seeds borne on an F_3 plant from a cross which he had made between "British Queen" (yellow wrinkled) and "Eclipse" (green round) ('Journ. R. Hort. Soc.,' vol. 28, pp. 3 and 4). These seeds included yellow rounds (Y R), yellow wrinkled (Y W), green rounds (G R), and green wrinkleds (G W). I sowed these four sorts separately. The Y R's gave either (i) plants bearing only Y R's, or (ii) plants bearing Y R's and Y W's, or (iii) plants bearing Y R's and G R's, or lastly (iv) plants bearing Y R's, Y W's, G R's, and G W's. The total number of yellow seeds, round and wrinkled, borne by plants falling in the last category (iv) was 232. These were sown, and 196 of them produced mature plants. 10 seeds from each of the 196 plants thus raised were preserved, in 2 packets of 5 from each plant. All of these seeds were sown this spring, except the contents of the 48 packets referred to in the text.

I started by writing down the catalogue-numbers of each of the packets. Then I recorded the nature of the grains in the seeds opposite the number of the packet containing them. Not until I had examined all the grains did I refer to my records for the characters (whether D D, D R, or R R in respect of seed shape) of the parent plants of the seeds in the packet.

I postponed this reference to my records until after all the grains had been examined, in order to prevent any possibility of the determination of the nature of the grains being influenced by a knowledge of the gametic constitution of the plant which bore them.

Our interest centres chiefly on the possible difference between the round peas borne on plants bearing both round and wrinkled, and those borne on plants bearing only round. I shall, therefore, postpone reference to the contents of the 12 packets containing wrinkled peas until after I have dealt with the rounds.

There were 36 packets containing round seeds. In every case—16—in which all the five seeds in a packet contained nothing but potato-shaped grains (and occasionally the very small compound grains found in "Eclipse") the plants bearing them had produced only round seeds. Table II gives the details of these families, and fig. 5 shows the appearance of these extracted dominant grains.* It will be seen that they do not differ sensibly from the *p*-grains of "Eclipse."

The small compound grains, which are often exceedingly minute, are likely to be overlooked: the absence of any record of them in the first four families in Table II may be due to the fact that I did not detect them because I was not on the look-out for them. The presence of these grains in a pea is indicated in the table by the letters *fc*. There was a single case (54.129.ii) of an apparently compound large *p*-grain divided longitudinally into two; but the rarity of such a grain points to the conclusion that this case was due to an accidental fracture; which would be quite likely to occur during the scraping of the soaked pea with a razor.

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* I have not measured any grains in F_5. But no difference could be seen, between the *p*-, *c*-, and *r*-grains in this generation and the grains, of these three types, already described.

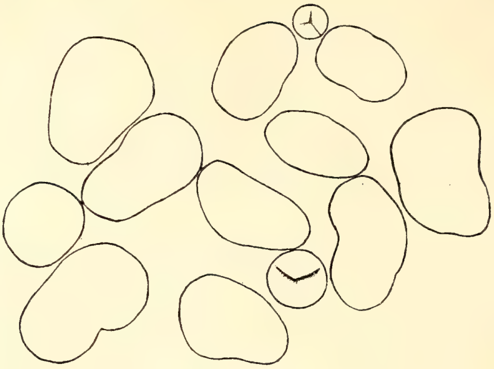


Fig. 5.—Starch-grains of DD Round Peas in F_5.

Table II.

Catalogue-number of parent-plant.	Number and characters of seeds borne by parent-plant.				Character of samples of seeds examined for starch-grains.	Nature of starch-grains in each of these seeds.				
	Colour.		Shape.							
	Y.	G.	R.	W.		i.	ii.	iii.	iv.	v.
54. 13	31	13	44		5 round	*p*	*p*	*p*	*p*	*p*
54. 17	27	14	38		„	*p*	*p*	*p*	*p*	*p*
54. 25	32	15	47		„	*p*	*p*	*p*	*p*	*p*
54. 50	20	8	28		„	*p*	*p*	*p*	*p*	*p*
54. 60	23	11	36*		„	*p*	*p*	*p*	*p*	*p fc*
54. 64	20	4	24		„	*p*	*p*	*p*	*p*	*p*
54. 83	19	11	30		„	*p*	*p*	*p*	*p*	*p*
54.104	31	14	45		„	*p fc*	*p*	*p fc*	*p*	*p*
54.117	40	8	48		„	*p*	*p*	*p*	*p*	*p fc*
54.129	30	15	45		„	*p*	*p*†	*p*	*p*	*p*
54.161	15	7	22		„	*p fc*	*p fc*	*p fc*	*p fc*	*p fc*
54.172	128	41	169		„	*p*	*p*	*p*	*p*	*p*
54.175	17	4	21		„	*p*	*p fc*	*p fc*	*p fc*	*p fc*
54.184	16	8	22		„	*p fc*	*p*	*p*	*p*	*p*
54.190	13	6	19		„	*p*	*p*	*p*	*p fc*	*p*
54.191	24	11	35		„	*p fc*	*p fc*	*p fc*	*p fc*	*p fc*

p signifies potato-shaped grain.

fc signifies the additional presence of the small compound grains found in "Eclipse," in very small numbers.

* When a discrepancy occurs in Tables II, III, and IV between the totals for cotyledon colour and totals for shape in a given plant, it signifies that in the case of some of the seeds of that plant the shape was determinable whilst the colour was dubious, or *vice versâ*.

† A large double grain in this pea.

In every case in which at least one of the five seeds in a packet contained either a round or an irregular round grain, the plants bearing them had produced both round and wrinkled seeds. That the category irregular round is not due to a difficulty to distinguish between *r*- and *p*-grain—even if it were possible for anyone familiar with these grains to have this difficulty—is shown by the fact that all peas containing grains falling into this category were borne by heterozygote plants, *a fact which was not known when the grains were being examined.* Table III gives the details of these families and fig. 6 shows some of the *r*-grains.

Table III.

Catalogue-number of parent-plant.	Number and characters of seeds borne by parent-plant.				Character of sample of seeds examined for starch-grains.	Nature of starch-grains in each of these seeds.				
	Colour.		Shape.							
	Y.	G.	R.	W.		i.	ii.	iii.	iv.	v.
54. 3	28	4	22	9	5 round	*r sc*	*p*	*p*	*p*	*r sc*
54. 18	20	9	17	6	,,	*r sc*	*p*	*r sc*	*r sc*	*r sc*
54. 19	31	12	31	12	,,	*p*	*p*	*r sc*	*p*	*p*
54. 23	26	6	22	6	,,	*r mc*	*r mc*	*r mc*	*r mc*	*r mc*
54. 33	26	10	26	9	,,	*i*	*r mc*	*r mc*	*r mc*	*p fc*
54. 52	33	13	30	13	,,	*i sc*	*p*	*p*	*p*	*r sc*
54. 57	23	8	21	10	,,	*i sc*	*i sc*	*i sc*	*i sc*	*i sc*
54. 65	20	3	15	8	,,	*p*	*r fc*	*p*	*i sc*	*p*
54. 77	30	9	35	2	,,	*p*	*r fc*	*r fc*	*r fc*	*p*
54. 89	32	9	29	8	,,	*r sc*	*i fc*	*r sc*	*p*	*r sc*
54.105	32	8	27	13	,,	*i mc*	*i mc*	*r mc*	*r mc*	*i mc*
54.112	27	6	16	5	,,	*p*	*i fc*	*p*	*p*	*p*
54.121	19	4	21	2	,,	*p*	*p*	*r mc*	*p fc*	*p fc*
54.146	15	4	14	5	,,	*p*	*p*	*p*	*p*	*r mc*
54.150	18	6	17	7	,,	*r sc*	*r sc*	*p*	*p*	*p*
54.177	26	7	25	8	,,	*r mc*	*r sc*	*r sc*	*r sc*	*r sc*
54.179	37	4	38	2	,,	*i fc*	*i mc*	*p fc*	*p fc*	*r mc*
54.180	36	11	38	9	,,	*p*	*p fc*	*i fc*	*p fc*	*p fc*
54.181	38	9	36	11	,,	*r sc*	*r sc*	*p fc*	*r sc*	*i fc*
54.182	37	9	30	13	,,	*r fc*	*r fc*	*i fc*	*r fc*	*r fc*

r = round grains (fig. 6).
i = irregular round.
sc = some compound.
mc = many compound.
Other symbols as in Table II.

The difference between peas marked *r mc* and those marked *r fc* is sometimes very great. A count of 508 grains from a pea marked *r mc* revealed 203 compound and 305 single. Whilst in a pea marked *r fc*, there were only 28 *c*-grains in 304 counted.

In the absence of any information as to the behaviour of the characters of the grains in F_2 it is not possible to make any definite statement as to the mode of segregation in this case; but the following points seem to be clear. We have seen that the grain in the F_1 seed is round. The evidence points to the fact that the heterozygote round peas in subsequent generations are characterised by the possession of *i*- or *r*-grains and homozygote rounds by *p*-grains. For, in the first place, no round grains appeared in 80 seeds from 16 plants which bore only round seeds. In the second place, of the 100 round seeds borne by plants bearing both round and wrinkled, 40 had *p*-grains as against the 33 or 34 expected by my theory—a discrepancy not so great that it cannot be accounted for by the smallness of the number of seeds.

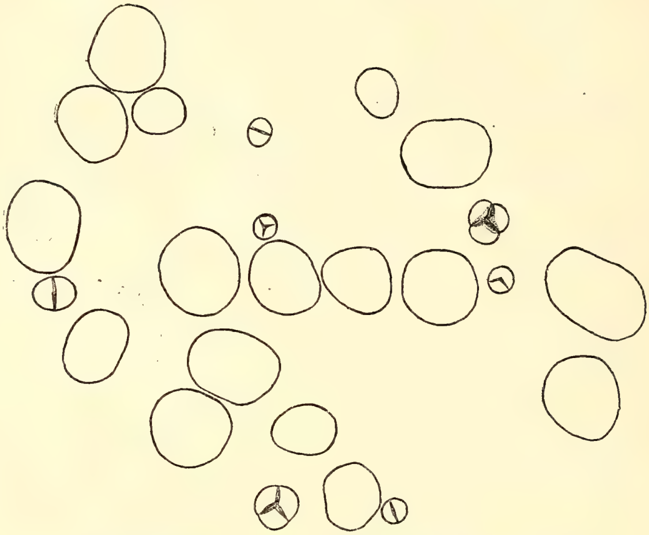


Fig. 6.—Starch grains of DR Round Peas in F_5.

If the association of *r*-grains with heterozygote round, and of *p*-grains with homozygote round holds good for the F_2 generation, we have a means of distinguishing between D D round and D R round in F_2; instead of, as at present, having to wait until their progeny are mature in the following year.

A further point is demonstrated by the nature of the grains in F_1, and borne out by those in F_5. It is that the shape of the grain is inherited separately from its composition—if we may use this term to cover the singleness or compoundness of the grain. In the round pea the grains are single and long; in the wrinkled they are compound and round; in the hybrid they may be either single or compound, but are more round than long. In a subsequent generation, F_5, we have *r*-grains exhibiting much compoundness (54.23) and others exhibiting little (54.182 i, ii, iv, and v). We have (possibly) *p*-grains either with no compounds, or with few; and intermediate grains either with few compounds (54.179 i) or with many (54.179 ii).

The wrinkled peas contained, as was to be expected, *c*-grains, but some of them contained in addition, very sparingly, *p*-grains, one of which is shown in fig. 7.

In the case of one plant, only one of the five seeds examined exhibited them. In the case of two other plants, two out of the five seeds exhibited them, and in the case of both these plants the five seeds had been noted, before soaking, as being only *rather* wrinkled.

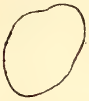


Fig. 7.—Example of occasional *p*-grain occurring, in extracted Wrinkled Pea in F_5.

The details of the grains and parentage of the wrinkled seeds are shown in Table IV.

Table IV.

Catalogue-number of parent-plant.	Number and characters of seeds borne by parent-plant.				Characters of samples of seeds examined for starch-grains.	Nature of starch-grains in each of these seeds.				
	Colour.		Shape.							
	Y.	G.	R.	W.		i.	ii.	iii.	iv.	v.
54. 38	24	7		32	5 *wr.*	*c*	*c*	*c*	*c*	*c*
54. 55	30	8		38	”	*c*	*c*	*c*	*c*	*c*
54. 59	18	3		21	”	*c*	*c*	*c*	*c*	*c*
54. 61	19	2		21	”	*c*	*c*	*c*	*c*	*c*
54. 66	27	3		30	”	*c*	*c*	*c sp*	*c*	*c*
54. 87	16	3		19	”	*c*	*c*	*c*	*c*	*c*
54. 97	32	12		41	5 rather *wr.*	*c*	*c sp*	*c sp*	*c*	*c*
54.113	14	3		17	5 *wr.*	*c*	*c*	*c*	*c*	*c*
54.127	11	8		19	”	*c*	*c*	*c*	*c*	*c*
54.138	30	15		45	”	*c*	*c*	*c*	*c*	*c*
54.140	24	6		30	”	*c*	*c*	*c*	*c*	*c*
54.187	13	7		20	5 rather *wr.*	*c*	*c sp*	*c*	*c sp*	*c*

c = compound grains.
sp = some *p*-grains.

The Amount of Water absorbed by the Seed on germination in the Various Generations in the Preceding Experiment.

Gregory's statement that, according to Denaiffe, "wrinkled seeds take up more water on germination than do round seeds," made me curious to find out how *much* more water wrinkled seeds take up than round; and, having determined this, to find out what relation the hybrid bore to its two parents with regard to this character.

I propose to refer to the capacity for taking up water as the absorptive capacity; and the measure which I shall use throughout of this capacity is the amount of water, taken up by a pea immersed in tap-water for 24 hours expressed as a percentage of the weight of the dry pea. The average absorptive capacity of 15 "British Queen" (*i.e.*, wrinkled) peas weighed separately is 122 per cent. The average absorptive capacity of 12 "Eclipse" (*i.e.*, round) seeds is 86 per cent. The absorptive capacity of a single F_1 seed, produced by crossing "British Queen" with "Eclipse," is 100 per cent. It will be seen that the hybrid is intermediate between the two parents.

The only evidence I have concerning the absorptive capacity in succeeding generations is derived from the examination of the contents of two sample pods in the F_2 generation derived from the cross (already referred to) between "British Queen" and "Eclipse," made by Mr. Wadsworth. The facts are shown in Table V. And it will be seen that whilst the difference between the absorptive capacity of round and wrinkled is well marked, that the absorptive capacities of the two kinds of rounds, *i.e.*, those with *p*-grains and those with *r*- or *i*-grains, is not in accordance with expectation. For in the pure round pea the *p*-grain is associated with an absorptive capacity 86, and in the F_1 round the *r*-grain with one of 100. And it was not unreasonable to suppose that the absorptive capacity was in some way determined by or, at any rate, associated with the nature of the grain rather than with the shape of the seed, for it is in seed-shape only that we find dominance; whilst in both grain-shape and absorptive capacity the two parent forms blend.

The expectation, however, suggested by this parallel between starch-grain and absorptive capacity in F_1 is not, as we see, fulfilled in F_2.

Table V.

Pod.	Individual seeds.	Colour and shape of seed.	Nature of grains.	Absorptive capacity.
89 19 16i	α	GR	*p*	96
	β	YR	*p mc*	100
	γ	GR	*p*	97
	δ	YW	*c*	141
	ε	GW	*c*	141
89 12 19ii	α	YR	*r mc*	100
	β	GR	*r mc*	97
	γ	YR	*p*	97
	δ	YR	*i mc*	97
	ε	YW	*c*	142
	ζ	GW	*c*	139

The r-*grains in other Hybrid Peas.*

The intermediate nature of F_1 grains was also observed in the following crosses (Table VI). The grains in these seeds were not measured; but it was evident, at a glance, that the grains were more round in some cases than in others. Thus, M 303 and M 313 had the most conspicuously round grains that I have seen in F_1 seeds.

Table VI.

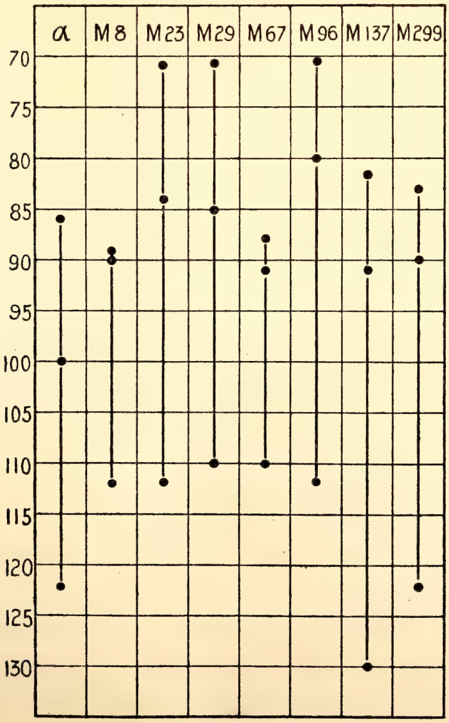
Catalogue-number of cross.	Number of seeds examined.	Pistil-parent.	Pollen-parent.
M 299	2	"British Queen"	Maple from Mr. R. H. Lock.
M 303	1	"	" Grubb, corn dealer, Oxford
M 312	1	"	Zucker Erbse. rotbl. kron.*
M 313	3	"	" "
M 316	1	"	Maple from Mr. R. H. Lock.

* A maple sugar pea with pink flowers and "mummy" (*i.e.*, fasciated) habit. Bought from Messrs. Haage und Schmidt, Erfurt, Germany (No. 1286, p. 24 in 1907 Catalogue).

The Absorptive Capacity in other Hybrids.

The absorptive capacity of the F_1 seed, as well as that of its two parents, was determined in eight cases altogether, one of which is "British Queen" by "Eclipse," to which reference has already been made. The results are shown on Table VII. The numbers at the left of the table refer to the absorptive

Table VII.



capacity. The uppermost dot in a column gives the absorptive capacity of the round parent in a cross: the lowermost dot, that of the wrinkled parent; and the intermediate one, that of the hybrid. The absorptive capacity is, in every case, except in that of the round parent in M 299, calculated from weighings made with the actual variety of round or wrinkled pea utilised in the cross; and, indeed, in many cases from the seeds from the very plant which served as mother or father. Instead of the absorptive capacity of the actual round parent in M 299 is written the average of the values for the five different varieties of round peas we have dealt with.

Table VIII gives the parentage of the crosses summarised in Table VII.

Table VIII.

Catalogue-number of cross.	Wrinkled parent.	Number of seeds weighed.	Round parent.	Number of seeds weighed.	Number of seeds of hybrid weighed.
a	♀ British Queen	15	♂ Eclipse	12	1
M 8	♀ Laxton's Alpha (Haage & Schmidt)	5	♂ Sangster's No. 1......	4	1
M 23	♀ " "	5	♂ Yellow round* (Genoa)	9	1
M 29	♀ Laxton's Alpha (Sutton)	8	♂ " "	9	1
M 67	♀ " "	8	♂ Bohnenerbse† (Haage & Schmidt)	3	1
M 96	♂ Laxton's Alpha (Haage & Schmidt)	5	♀ Yellow round (Genoa)	9	6
M 137	♀ Telephone (Haage & Schmidt)	3	♂ *Pisum arvense*‡ *hibernicum*	6	1
M 299	♀ British Queen	15	♂ Maple from Mr. R. H. Lock	*vide* text	2

* Plants grown from (evidently unimported) yellow seeds which I bought in a small shop in Genoa.

† A large, long pea with a black hylum, which, however, is not situate in the middle of one side as in the bean, but at the end.

‡ *Pisum arvense hibernicum* is a small pea seldom attaining 2 feet in height, though it is not dwarf in habit, sold by Messrs. Haage und Schmidt (No. 1660, p. 30, 1907 Catalogue). Its seeds can be sorted into four categories. Maple and purple spots on grey: maple on grey; purple spots on grey, and grey. *Vide* "Report II to Evolution Committee of Royal Society," and R. H. Lock, 'Roy. Soc. Proc.,' B, vol. 79, *supra*, p. 28.

Summary.

My investigations on this subject are being continued. The facts so far brought to light are:—

1. That, although roundness is dominant over wrinkledness in peas, the starch-grain of the F_1 generation (the round or *r*-grain—fig. 4) is a blend between the type of grain of the round pea (the potato-shaped or *p*-grain—

fig. 1) and the type of grain of the wrinkled pea (the compound or *c*-grain—fig. 2), in respect of three characters—

(*a*) It is intermediate in shape as measured by its length-breadth index—that of the *p*-grain being 66, that of the *c*-grain 92, and that of the *r*-grain 85 (neglecting decimals).

(*b*) It is intermediate in the distribution of compoundness, inasmuch as some of the *r*-grains are compound and some single.

(*c*) It is intermediate in the degree of compoundness, inasmuch as amongst those *r*-grains which are compound the most usual number of constituent pieces is three, whereas in *c*-grains it is six.

2. In a subsequent generation—F_5—the homozygote round peas contain *p*-grains; the heterozygote round peas contain *r*- or intermediate grains. But both *r*- and intermediate grains may be associated either with a high, or with a low degree of compoundness.

3. *P*-grains occasionally occur in wrinkled peas in F_5, and the evidence (Table IV, 54.97 and 187) suggests that the existence of these grains in wrinkled peas tends to make them less wrinkled.

4. A wrinkled pea takes up more water when it germinates than a round one. The hybrid between a round and a wrinkled pea is intermediate in respect of this character between its two parents.

5. But this intermediateness of the hybrid in absorptive capacity is not occasioned by the intermediateness of the starch-grain of the hybrid, because, in F_2, peas containing *r*-grains *and peas containing* p-*grains* both have the same absorptive capacity as the F_1 pea (see Table V).

6. When, therefore, we cross a round with a wrinkled pea, we are dealing with *four* separately heritable characters :—

(i) The shape of the pea—whether round or wrinkled.
(ii) The absorptive capacity of the pea—whether low or high.
(iii) The shape of the starch-grain—whether long or round.
(iv) The constitution of the starch-grain—whether single or compound.

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Localisation of Function in the Lemur's Brain.

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[Plates 2—4.]

The brain of the Lemur, the lowest of the ape-like animals, does not appear to have been subjected previously to a thorough examination. Page May and Elliott Smith brought a brief communication on the subject before the Cambridge Meeting of the British Association in 1904.* Their experiments were apparently limited to stimulation of the cerebral cortex, and they have never published a full account of their work. Brodmann† has worked out some of the histological details of the structure of the cortex cerebri, and Max Volsch‡ has performed a stimulation experiment upon one Lemur. The work of these investigators will be referred to again in the course of this paper.

Our own investigation has in the main dealt with the motor centres, and the experimental methods adopted have been the usual ones of stimulation and extirpation. In animals so low in the scale, stimulation is to be regarded as the more decisive of the two methods for the purpose of localisation. The extirpation experiments have, however, confirmed the results of stimulation, and in these experiments the course of the resulting degeneration was followed by histological examination of the brain and spinal cord. The results, moreover, agree remarkably closely with those obtained by a study of the histological structure of the various regions of the cortex cerebri.

One of us (W. D. H) is responsible for the experimental part of the investigation which was carried out at King's College, London; the other (F. W. M.) is responsible for the histological portion. We have to thank Miss Agnes Kelley for assistance in the histological work, and also for the drawings which accompany this paper.§

The species of Lemur employed was the Ring-tailed Lemur of Madagascar (*L. catta*) except in two experiments where the Black Lemur (*L. macaco*) was used. The results obtained in both species were practically identical.

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* British Association Reports, 1904, p. 760.

† 'Journ. f. Psychologie u. Neurologie,' vol. 6, p. 272, 1906.

‡ "Ein Rindenreizungsversuch an einem Halbaffen," 'Monatsschrift f. Psychiatrie u. Neurologie,' 1906.

§ A full description of the histology of the cerebral cortex of the Lemur will be published later by one of us (F. W. M) in conjunction with Miss Kelley. In the present paper only the motor area will be considered.

Stimulation Experiments.

These were five in number. In all cases the anæsthetic employed was ether, and anæsthesia was maintained until the animal was killed at the conclusion of the experiment by an overdose of chloroform.

The brain was exposed in the usual way, care, of course, being exercised not to injure its surface, nor to wound any large vessels. Its surface was explored by the usual two-point platinum electrodes connected to the secondary coil of a du Bois Reymond's inductorium. The primary circuit was arranged for faradisation and included one cell. The approximation of the secondary to the primary coil was such that the current as tested by the tongue of the operator could be felt as a little more than a faint tickling. In some experiments Sherrington's unipolar method* was used. Professor Sherrington was good enough to furnish us with one of his own electrodes for the purpose. The results obtained by this method were identical with those obtained by the two-point electrodes. It did not appear to us so easy to evoke movements by the single electrode, probably because the number of cortical cells excited is smaller than when two are used. The double-point electrodes, moreover, possess the advantage of being so readily tested in relation to strength of current by application to the operator's tongue.

Short protocols of the five experiments performed are as follows:—

Lemur 1. *Lemur catta.*—Right hemisphere exposed in frontal and central regions, and explored with single-point and double-point electrodes. Resulting movements noted.

Lemur 2. *Lemur catta.*—Experiment on Lemur 1 repeated, and some details of map made at the first experiment filled in and corrected. At the conclusion of this, the posterior part of the hemisphere was exposed, and its posterior pole excited; no definite eye or other movements were thereby elicited.

Lemur 3. *Lemur macaco.*—The same experiment repeated; in this case the excitable area did not extend quite so far posteriorly as in previous experiments.

Lemur 5. *Lemur catta.*—Stimulation of the occipital region, even with stronger currents than those usually employed, produced no effects, although stimulation of the anterior eye centres produced eye and head movements as in previous experiments. The occipital region on both sides was investigated.

Lemur 6. *Lemur catta.*—Confirmatory experiment, the special object of which was to determine the posterior limit of the excitable area. No movements were evoked by stimulation of occipital regions, although these were better exposed than in previous experiments and explored on all surfaces.

General Results of Stimulation Experiments.

The convolutional pattern of the brain, as will be seen by looking at the accompanying figure (Plate 2, fig. 1), is a simple one.

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* As described in a paper by Fröhlich and Sherrington, 'Journ. of Physiol.,' vol. 28, p. 14, 1902.

The diagram represents the cerebrum as seen from above. The fissure of Sylvius is easily recognisable; beneath this is a well-marked parallel fissure, and above it is the sulcus known as the lateral fissure. In the frontal region the best marked fissure is the sulcus rectus. On the mesial surface of the hemisphere (not shown in the diagram) the intercalary fissure and the calcarine fissure are the most prominent sulci.

An interesting morphological point is, which of the smaller fissures in the fronto-parietal region corresponds with the central fissure (fissure of Rolando) of higher animals. These are four in number, and are labelled 1, 2, 3, 4 in the diagram. It will be noticed that these differ in length, and to some extent in position in the two hemispheres; the drawing of the right hemisphere, however, more accurately represents their average size than the left. Fissure 4 is too far forward, and fissure 3 is too far back for the Rolandic fissure; and our experiments have given us no certain answer as to whether fissure 1 or 2 is the fissure of Rolando, for the excitable area extends behind both.

Sherrington and Grünbaum have shown that, in the higher apes, the excitable cortex does not extend behind the Rolandic fissure. In one experiment we have performed on one of the lower apes (*Macacus rhœsus*), we have found by the unipolar method the same to hold. So far as physiological enquiry can give any reply to morphological homologies, we feel inclined to regard fissure 2 rather than fissure 1 as the representative of the central sulcus, since behind it movements are evoked with greater difficulty than in front of it.

The precise posterior boundary of the excitable cortex is very difficult to determine with accuracy, and is variable in different animals. Our diagram (Plate 2, fig. 1) represents the extreme posterior boundary, but in one or two instances it was somewhat more anterior. The cause of the greater difficulty of evoking movements here becomes clear on histological examination; the large motor cells become more scattered as one passes backwards. We obtained the most energetic movements from this posterior region by placing the electrodes 3 mm. apart, and applying them in a longitudinal direction. Very marked results (extension of arm and fingers) were in this way obtained from the oval area so marked in the figure. This may be explained on the hypothesis that the series of psychomotor cells presiding over this particular movement are arranged in a longitudinal direction, so that when the electrodes are applied in the manner stated the current spread over the whole of the excitable area of cortex representing the extension of arm and fingers.

The diagram represents better than any amount of verbal description the disposition of the motor centres. The trunk and, more posteriorly, the leg areas are uppermost, and extend on to the mesial surface as far as the intercalary fissure. Below this and in front are the various head and face areas. The areas controlling the upper limb are posterior to, and somewhat higher than these.

The excitation experiments show that, as in the primates, there exist in the Lemur three areas related to lower limb, upper limb, and head region respectively, from above downwards, in inverse order therefore to the spinal arrangement. Moreover, they substantiate and accord with the ablation experiments, which will be described later.

The large size of the hand and finger area is not remarkable in view of the habits of the Lemur. It does not pick up its food with the hands in the same way in which a monkey does, but as a rule uses the hands to support the morsel which is picked up by the mouth. The hand movements are, however, undoubtedly more highly developed than in quadrupeds, and come chiefly into play in climbing. There is a well-formed and opposable thumb, which must be extremely useful in the arboreal life of the animal. We were unable to discover any special thumb area in the cortex.

In the area marked "upper limb mainly shoulder," the movements which occur spread to the lower part of the limb and the digits on keeping up the usual weak stimulation, or by the use of a somewhat stronger current; the resulting movement is a primary one of progression. The same is true for the lower limb, especially in those centres which, in the first instance, control movements of its upper segment. This spreading does not occur to any noticeable degree in the head, face, eye and ear movements. This, again, is explicable on histological examination, for the Betz cells in this region are much smaller than in the limb areas, and so do not possess so many connections with other cells. The difference is so marked that we have considered it worth while to contrast, in Plate 4, the two types of motor cortex.

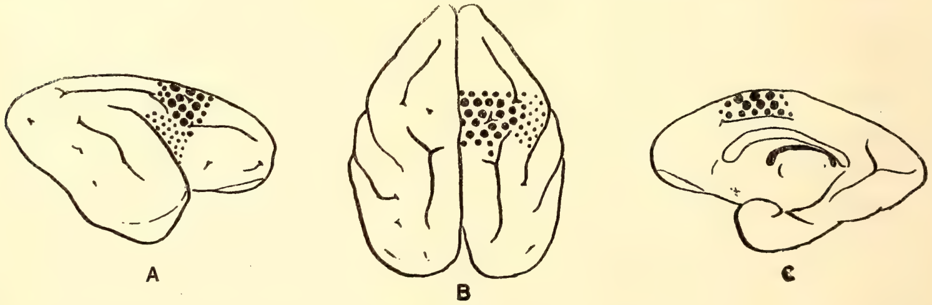
It will be noticed in Plate 2 (fig. 1) that the head, face, and tongue area passes on to the outer side of the sulcus rectus. The extent to which this occurs probably varies in different animals, but in those subjected to microscopic study the Betz cells did not extend beyond the outer lip of the sulcus. It is probable that this is a more trustworthy guide than stimulation, for in the latter method it is often difficult to entirely eliminate spread of current.

In the large area marked "Pricking of ear" on the outer side of the anterior end of the lateral fissure, and which is also characterised by Betz cells of the small type, the shaded area near the Sylvian fissure was in some experiments the part, stimulation of which elicited the most marked movements; this is quite intelligible because in this part one is approaching the auditory centre, which analogy would teach us to place on the opposite side of the Sylvian fissure. This, however, did not obtain in all cases, and in one experiment the area passed upwards so as to slightly overlap the lateral fissure.

As already noted in the protocols, repeated attempts to evoke eye movements from the occipital pole led to entirely negative results. It is quite certain that this region is, as in other animals, the visual area, for histological examination of this part of the brain shows it to possess a well-marked line of Gennari, and the other structural features of the visual cortex. It is quite possible that if we had been able to stimulate in the depth of the calcarine fissure, where the solitary cells of Meynert are more abundant, we might have obtained eye movements.

The Histology of the Excitable Area.

It is a comparatively easy matter to map out the area containing the large Betz cells; moreover, although, as in the primates, the region of cortex behind the motor area contains large pyramidal cells with Nissl granules, yet this can be differentiated from the motor area proper by the existence of a granular layer, a sure sign of sensory function. Fig. 1, Plate 4, shows the type of cortex with the giant Betz cells; fig. 2, on the same plate, shows the type of cortex with the smaller Betz cells. The accompanying drawings in the text indicate the extent and boundaries of these two types, the size of the Betz cells being roughly shown by the size of the dots.



Diagrams A, B, and C are drawings of the outer and mesial surfaces of one hemisphere (A and C), and a drawing of the cerebrum as seen from above (B). The dotted area is the portion characterised by the possession of Betz cells, the size of which is roughly indicated by the size of the dots.

The first type of cortex (with large Betz cells) covers that part of the outer and mesial surface which lies between the posterior end of the sulcus rectus, the anterior end of the sulcus lateralis, and the intercalary sulcus. It is continued further forward within the sulcus rectus than can be indicated on surface diagrams. Anteriorly and posteriorly it gradually merges into the types of cortex which are respectively found in frontal and post-central regions.

The second type of cortex (with smaller Betz cells) covers the area which lies between the extremities of the sulcus rectus and sulcus lateralis, and extends downwards as far as the superior wall of the Sylvian fissure. Superiorly it merges into the first type of motor cortex; anteriorly into an area intermediate to this type and the type of the frontal cortex proper; and posteriorly into one intermediate to this type and the temporal and post-central types.

Further histological details of these two types are as follow:—

First type of motor cortex. (Plate 4, fig. 1).—The cortex is about 2 mm. deep; the depth of the molecular layer is 0·17 mm., that of the pyramidal layer and granules about 0·9 mm.; that of the pallid zone in which the Betz cells lie about 0·2 mm.; and that of the polymorph layer about 0·7 mm. The pyramidal cells are larger and have more processes than in other parts of the cortex (those of the post-central and temporal types most nearly approaching them in form). They are somewhat irregularly arranged, owing, perhaps, to the presence of the processes of the Betz cells. Granules are scattered in fair numbers at the bottom of the pyramidal layer, but they do not form a distinct layer. The infra-granular pyramids are the most typical feature of this area. They are, for the most part, well-formed giant pyramids (Betz cells), containing Nissl bodies and having several branched processes. They frequently measure as much as 60 μ by 25 μ, and are sometimes larger. This line of cells occupies a pallid zone in which only a few other cells are scattered, these being smaller Betz-like cells, faintly stained pyramidal cells, and a few granules. Some of the large Betz cells closely resemble the typical giant Betz cells of the cortex of the higher apes, but many are more pyramidal in shape. The tendency to arrangement in nests, which has been described in the human cortex, is not general, though it can sometimes be seen. The largest Betz cells are found immediately before and behind the small fissure (No. 1 in Plate 2, fig. 1), which lies in the middle of this area, and between it and the intercalary sulcus.

Second type of motor cortex (Plate 4, fig. 2).—The pyramidal cells are smaller than in the first type. A line of darkly staining stellate cells is scattered above or among the granules, and the *granules form a fairly well marked line.* The cells which correspond in position to the Betz cells in the first type of motor cortex are not conspicuous either in size, shape, or number. But a somewhat scattered line of cells can be seen, measuring about 25 μ to 35 μ by 15 μ, which are Betz-like in shape, having many branched processes staining more deeply than the other cells and possessing Nissl granules. The results of excitation and ablation of this area support the inference that this area is motor in function, although it differs considerably from the typical giganto-pyramidal motor cortex in the characteristics mentioned above. *The definite layer of granules would suggest that this cortex is sensori-motor in function like the primary visual cortex of the calcarine region.* This is best marked in the region from which ear movements were elicited.

Brodmann* has also mapped out the distribution of the giant pyramids in the Lemur's brain, and his results correspond fairly closely with ours. He points out that the little dimple (our sulcus 1 in Plate 2, fig. 1) is inconstant in position, and does not agree with

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* *Loc. cit.*

Ziehen, that it is homologous with the sulcus centralis of the primates, for the area giganto-pyramidalis extends behind it. In support of this argument he has also examined the brains of two monkeys, *Periodictus potto* and *Propithecus coronatus.* The former has a well-marked central sulcus, and this is the caudal boundary of the area giganto-pyramidalis, as well as of the excitable area. In Propithecus, the central sulcus is represented by a small dimple only, very like what we have labelled sulcus 1 in the Lemur. In Propithecus, however, it forms the caudal boundary to the excitable area, and to the area giganto-pyramidalis. He therefore argues that in this monkey it is the true homologue of the central fissure, and that the apparently similar dimple in the Lemur's brain cannot be the same, since it lies within the excitable area and the area of large Betz cells.

Max Volsch's* work was apparently undertaken to prove the correctness of Professor Ziehen's view. He concludes that the general arrangement of the motor centres appears to agree with that of the primates, and that they are situated in front of the small sulcus we are discussing, and which he therefore regards as the representative of the central fissure. He, however, wisely remarks that the results of only one experiment cannot lead to far-reaching conclusions.

Flatau and Jacobsohn† regard the anterior end of the lateral fissure as homologous with the central fissure, and look upon the little sulcus (1) as a rudimentary pre-central sulcus.

Extirpation Experiments.

The extirpation experiments were four in number. The operations were performed under ether anæsthesia, and with strict antiseptic precautions. Healing took place by the first intention in all cases ; the paralysis exhibited after the operation was not pronounced in any experiment, and soon passed off; this transitory nature of the paralysis has been frequently noticed before, especially in animals low in the scale. The animals were finally killed by chloroform about a fortnight after the operation, and the parts which were to be subjected to microscopic study were then placed in suitable preservative fluids.

The following are short protocols of the four experiments performed :—

Lemur 4. *Lemur macaco.*—The motor area was removed as completely as possible on the right side, except that the part below the sulcus rectus and lateral fissure, which governs mainly head, eye, and ear movement, was left intact. Immediately after the operation there was conjugate deviation of head and eyes to the right, and the left pupil was rather larger than the right. These effects were transitory. The paralysis of the left limbs had passed off to a great extent two days later ; the fingers and toes on this side were, however, not so well used in climbing as those on the right side. The animal was killed by chloroform 14 days after the operation, by which time it was difficult to recognise any paralysis. The grip of the left hand was not noticeably weaker than that of the right.

Lemur 7. *Lemur catta.*—A large amount of the motor area was removed on the right side. The extirpated portion was principally the area for the upper limb. Paralysis of the opposite side was marked immediately after the operation, but eventually passed off in the way described in the experiment on Lemur 4. The animal was killed 14 days after the operation.

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* *Loc. cit.*

† 'Handbuch der Anatomie u. Vergl. Anat. des Zentral-Nervensystems der Säugethiere,' 1899.

Lemur 8. *Lemur catta.*—The leg area was removed on the right side. A good deal of hæmorrhage occurred during the operation from the longitudinal sinus, a large branch of which was injured and had to be tied. The animal, nevertheless, made a good recovery, and paralysis of the leg had largely disappeared by the time the animal was killed, 14 days later.

Lemur 9. *Lemur catta.*—Here the operation was limited to removal of the excitable (mainly ear, eye, and face region) area on the outer side of the lateral sulcus and sulcus rectus in the right hemisphere. No paralysis was noticeable ; indeed, after recovery from the anæsthetic, the animal was climbing about the room, and eating fruit heartily within an hour of the operation. It was killed 12 days later.

It will be noticed that the last three operations form a series in which the arm area, the leg area, and the head and face area (characterised by the small Betz cells) were respectively removed. We present, in the accompanying plates, diagrams of the degeneration tracts, selecting, of course, only a few important regions from the large number of sections which were made. It will be seen that, although the lesions were not absolutely limited to arm, leg, and face areas respectively, yet from our knowledge, derived from stimulation, we were able fairly accurately to perform the operations we have indicated.

In each case the large drawings which come first are those of the brain, drawn on a somewhat smaller scale than the others. This shows the position of the lesion, and also the degenerated fibres (indicated by black dots and lines), passing in three directions : (1) to the internal capsule ; (2) to the opposite hemisphere by the corpus callosum ; and (3) to neighbouring convolutions by association fibres. The smaller drawings show the degenerated tracts in lower portions of the central nervous system.

Histological Examination.

The brains and spinal cords were placed in Müller's fluid for three weeks ; appropriate slices 3 to 4 mm. in thickness were then placed in Marchi's fluid for 14 days, the fluid being changed several times. After imbedding in celloidin, sections were then prepared.

The Resulting Degeneration.—In all cases there was : (1) A heavy degeneration of the fibres of the internal capsule on the side of the lesion, which could be traced to the *crusta* of the *pedunculus cerebri.* (2) A degeneration much less marked—with the degenerated fibres not so coarse—of the corpus callosum ; most of these enter the radiating fibres of the opposite hemisphere, and pass especially to that portion which corresponds to the region of ablation; some few may pass into the internal capsule (*vide* figs. 1 and 2, Plate 3). The degenerated myelin, in the form of black dots and rods, cannot be followed farther than the deeper layers of the opposite cortex. Apparently these fibres either end in the polymorph layer or the inner line of Baillarger, where the large psychomotor cells are situated (*vide* fig. 2, Plate 3). (3) A large number of coarse and fine degenerated association fibres pass from the lesion to the adjacent uninjured motor cortex; some run horizontally into the inter-radial association fibres, the majority, however, enter the radiating fibres. We were uncertain whether degenerated association fibres pass to remote regions of the cortex, but some certainly pass to the post-central and temporal regions; there was no degeneration in the prefrontal, hippocampal, or occipital regions. (4) The basal ganglia, especially the nucleus caudatus, exhibited great numbers of scattered fine black dots; it is not improbable that this may be accounted for by degenerated collaterals given off by the degenerated efferent fibres contained in the internal capsule.

The degeneration in the lower portions of the central nervous system may be most conveniently described by taking separately the individual cases.

In Lemur 9 (Plate 3, figs. 1 to 8) the lesion involved the head and face region. The degenerated peduncular fibres were traced into the crusta of the mid-brain (fig. 3); they are most numerous in its outer and inner fourth, and least numerous in the middle two fourths. In Lemurs 7 and 8, where limb areas were removed, this relationship was reversed. In Lemur 9, as might be expected, degenerated fibres can be seen leaving the pyramidal bundles in the pons, and crossing the median raphe, to arrive at the various motor nuclei of the pons, *e.g.*, the facial and fifth. This decussation of degenerated fibres can be followed all the way down the pons and medulla. Only a few degenerated fibres exist in the crossed pyramidal tract of the spinal cord in the upper cervical region. (See figs. 3 to 7, Plate 3.) These fibres have entirely disappeared in the cervical enlargement (fig. 8).

In Lemur 7 (Plate 2, figs. 2 to 8), the lesion involved mainly the region of the forelimb, and we observe a heavy scattered degeneration in the pyramidal system of fibres in the pons and medulla; these fibres do not begin to decussate until the mid-portion of the medulla is reached, that is, they decussate at a much lower level than in Lemur 9, and at a higher level than in Lemur 8. There are no degenerated fibres in the lumbo-sacral region. (Fig. 8.)

In Lemur 8 (Plate 3, figs. 9 to 16) the lesion has involved a part of the arm area and the whole of the area of the trunk and lower limb, except that lying on the mesial surface of the hemisphere; we observe the heavy pontine and medullary degeneration, but the fibres do not begin to decussate until a lower level in the medulla is reached than in Lemur 7, and they can be followed all the way down the cord to the lumbo-sacral region, as shown in figs. 14, 15, and 16. There is, indeed, almost as heavy a degeneration in the crossed pyramidal tracts of the cervical region (fig. 14) as in the pons and medulla. Moreover, the degenerated fibres have not left the crossed pyramidal tract in the mid-dorsal region, for there are nearly as many degenerated fibres seen in the sixth dorsal segment (fig. 15) as in the eighth cervical (fig. 14). Most of the degenerated fibres can be seen leaving the pyramidal tract in the lumbo-sacral region; when fig. 16 (section at the fifth lumbar level) is compared with fig. 15 (section at the sixth dorsal level), a great diminution in the number of degenerated fibres is seen.

We have not presented any diagrams of our examination of the central nervous system in the case of Lemur 4. It was thoroughly examined, and the results were confirmatory of those found in the other cases. Lemur 4 belonged to a different species from the other three, but this makes no difference to the course of the degeneration. In this case, also, the lesion was more extensive, including both arm and leg cortical areas, so the degeneration was more extensive, and showed practically a combination of the results noted in Lemurs 7 and 8.

In no case were we able to follow degenerated fibres to the anterior horn; in some sections they could be seen lying as small compact bundles within the base of the posterior horn.

As in the lower apes, there is no direct pyramidal tract in the spinal cord of the Lemur, although it may be mentioned that one finds generally a few (homo-lateral) degenerated fibres in the crossed pyramidal tract of the same side as the lesion.

Summary.

1. The brain of the Lemur has a simple convolutional pattern, and the fissures are few and for the most part shallow.

2. The motor areas are limited to the central region of the cortex, and details of the localisation by the method of stimulation are given in Plate 2 (fig. 1).

3. Extirpation of the excitable areas is followed by transitory paralysis of the corresponding regions on the opposite side of the body, and by degeneration of the tracts which pass to the bulbar or spinal grey matter which controls these movements. Degeneration also occurs in commisural (callosal) and association tracts in the cerebrum.

4. The motor areas are characterised histologically by the presence of Betz cells. Localisation by histological study is therefore possible, and the close

correspondence of the results so obtained with those obtained experimentally is well seen by comparing together the figures in the text with Plate 2 (fig. 1).

5. There are, however, two types of motor cortex in the Lemur's brain, and these are shown side by side in Plate 4. The large type of Betz cell is found in the greater part of the motor cortex, particularly where limb and body movements are represented. The smaller type of Betz cell is found in the area governing face, tongue, ear, and eye movements, and in this excitable region there is a layer of granules; it is therefore probably sensori-motor.

6. Although the investigation relates in the main to motor representation, histological examination of the occipital (and especially calcarine) region shows it to possess the structural characters of the visual cortex in other animals. That no eye movements could be elicited by faradic stimulation of this region is probably due to the difficulty of the experiment, as explained in the text.

The expenses of this research have been in part defrayed from grants made to us by the Government Grant Committee of the Royal Society, and by the Science Committee of the British Medical Association.

DESCRIPTION OF PLATES.

Plate 2.

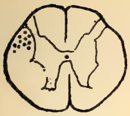
Fig. 1.—This is a map of the Lemur's cerebrum from above. The names of the principal sulci, and the position of the subdivisions of the motor area, as ascertained by the stimulation method, are shown on the right hemisphere. The homologies of the small sulci, marked (1) and (2), are discussed in the text.

Figs. 2—8.—These show a series of drawings made from the central nervous system of Lemur 7, in which the upper limb area was removed. The course of degeneration is indicated by black lines and dots.

Fig. 2 is a section of the injured right hemisphere. The position of the lesion is indicated by a dotted line, and degenerated fibres are seen passing in three directions, namely, by the corpus callosum to the opposite side, by association tracts to adjacent convolutions, and by the internal capsule to the cerebral peduncle.

Figs. 3, 4, 5, and 6 are sections at various levels of the pons and bulb. Decussation of degenerated fibres begins at a lower level than in the case of Lemur 9 (in which the head area was removed, Plate 3, figs. 1—8), and at a higher level than in the case of Lemur 8 (Plate 3, figs. 9—16), in which the leg area was removed.

Fig. 7 is a drawing through the thoracic region of the spinal cord; a few degenerated fibres are still seen in the crossed pyramidal tract, but these have entirely disappeared in the lumbar region (fig. 8).



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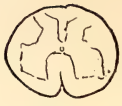

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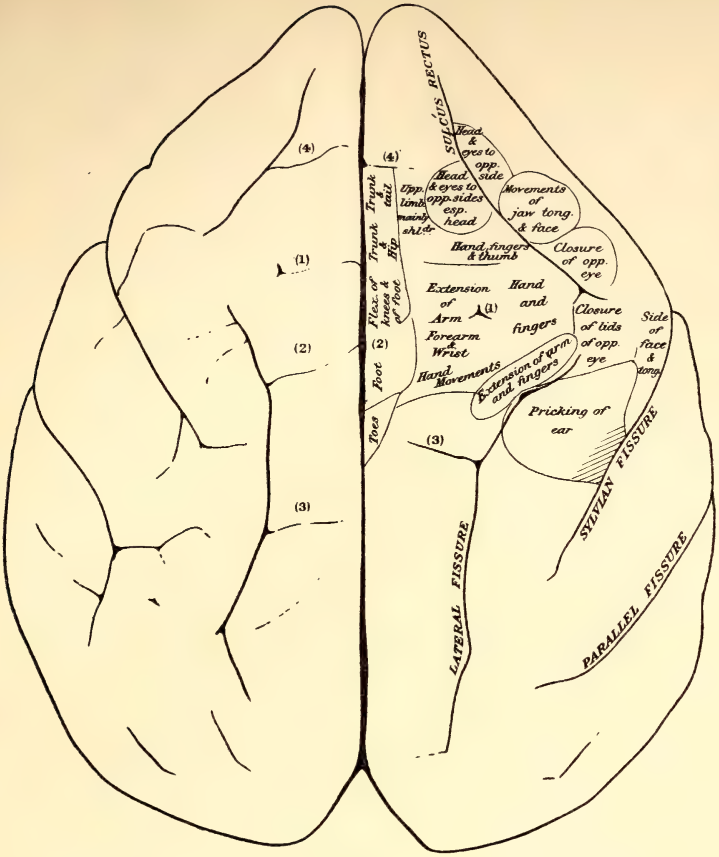
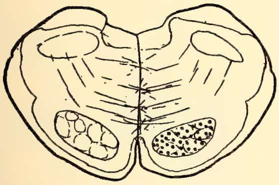

(4)
(1)
(2)
(3)
SULCUS RECTUS
(4)
Trunk & tail
Upp. limb mainly shl.dr
Head & eyes to opp. sides esp. head
Head & eyes to opp. side
Movements of jaw tong. & face
Trunk & Hip
Hand, fingers & thumb
Closure of opp. eye
Flex. of knees & of foot
Extension of Arm
(1)
Hand and fingers
Closure of lids of opp. eye
Side of face & tong.
(2)
Forearm & Wrist
Foot
Hand Movements
Extension of arm and fingers
Pricking of ear
Toes
(3)
SYLVIAN FISSURE
LATERAL FISSURE
PARALLEL FISSURE

1



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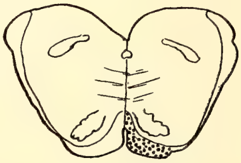

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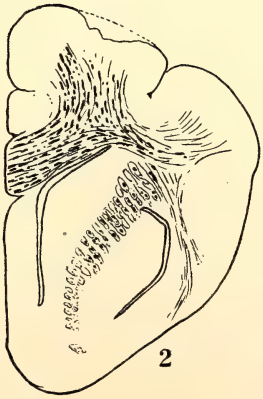

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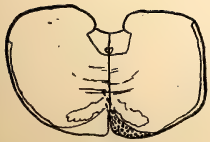

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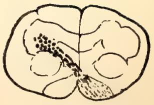

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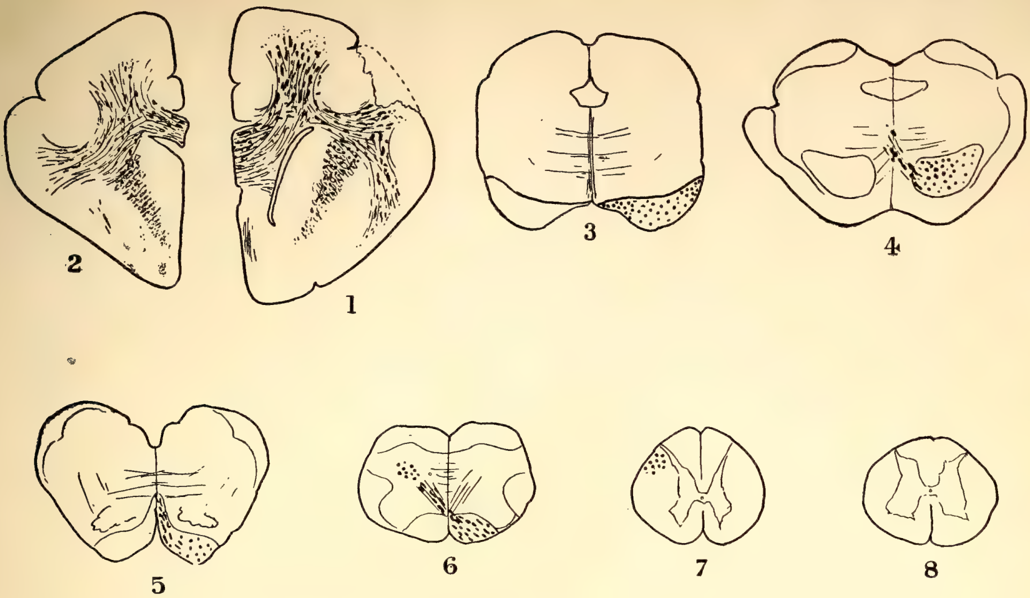
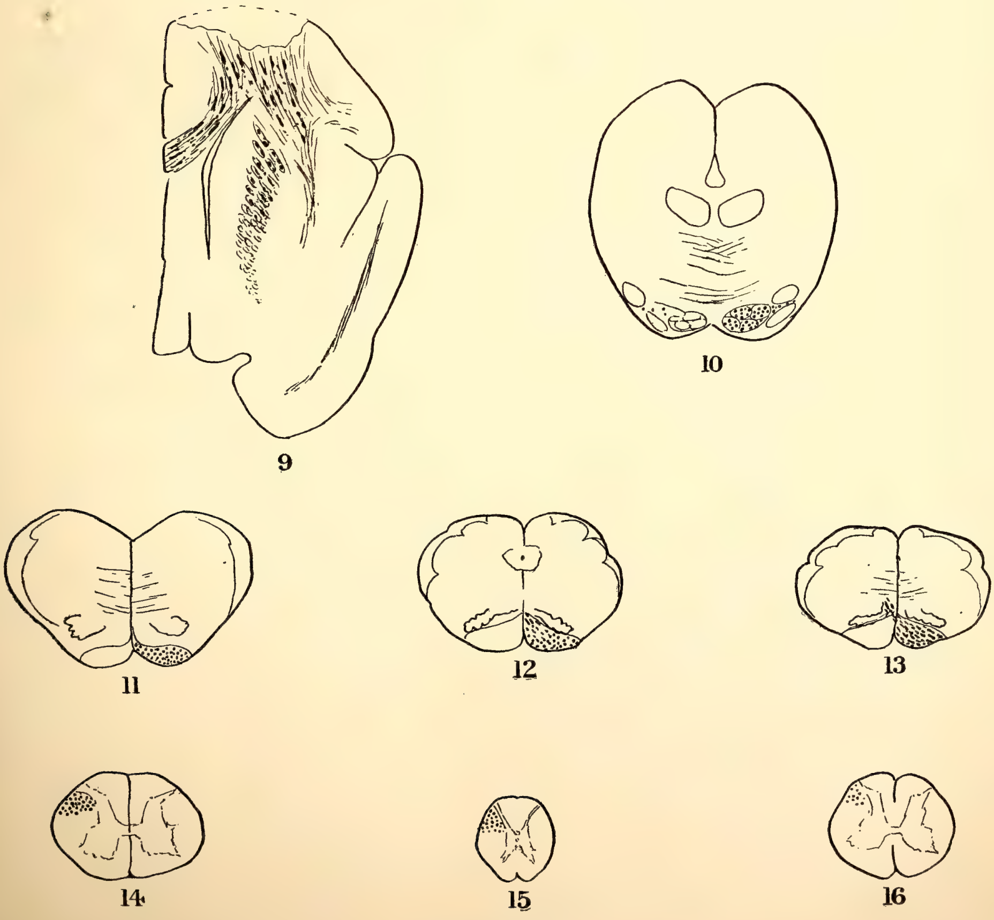
LEMUR 7.



Lemur 9.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Lemur 8.

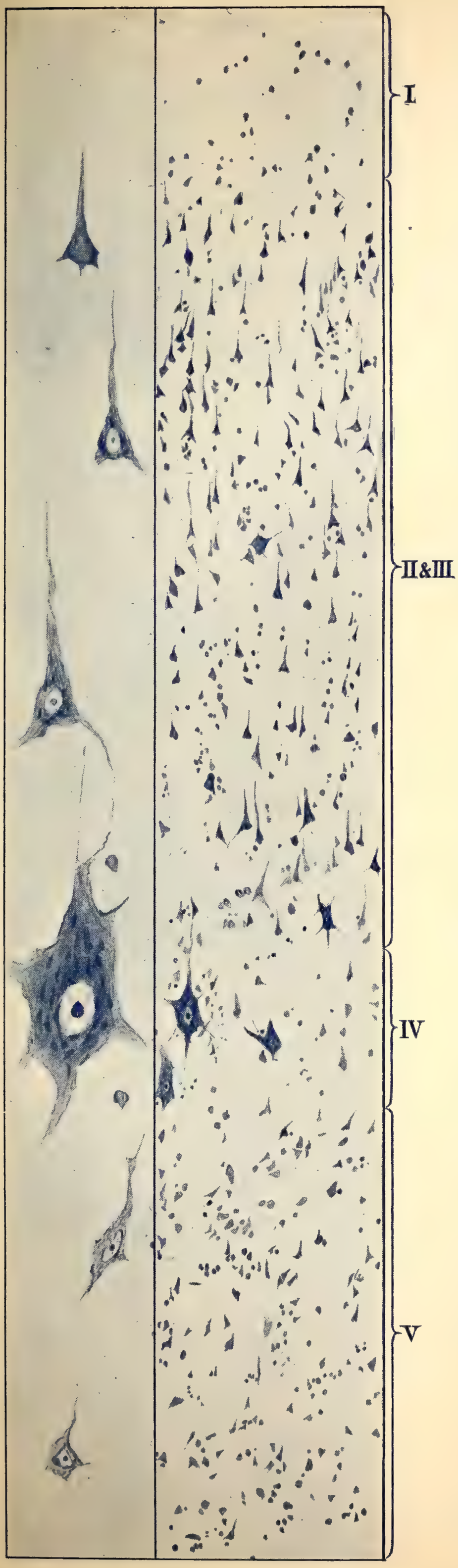

Fig. 1.

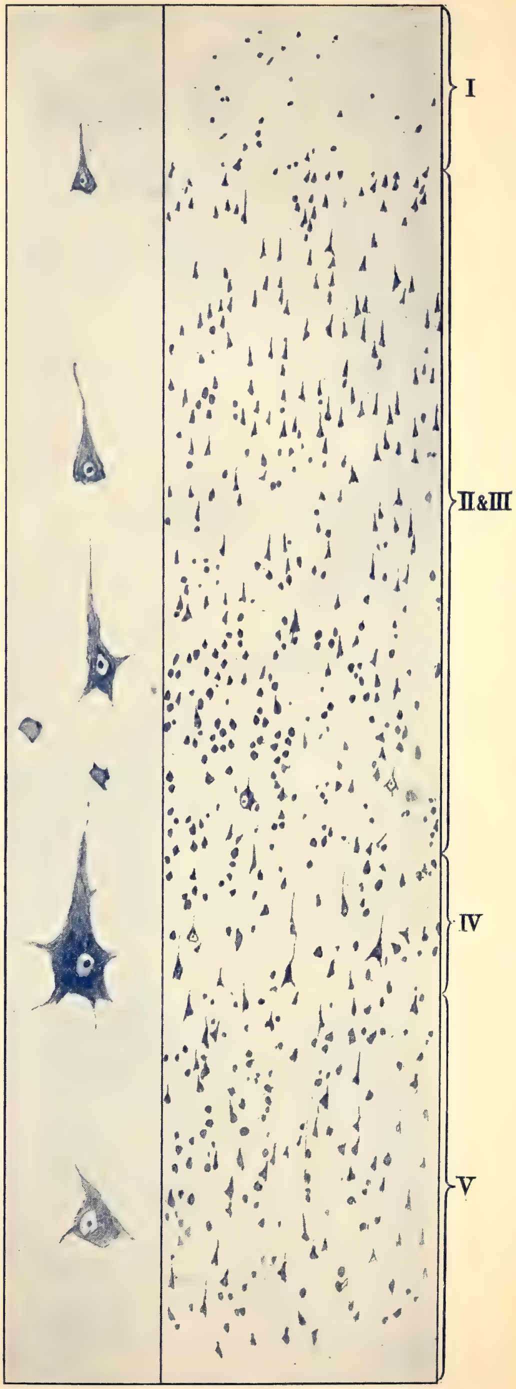

Fig. 2.

Plate 3.

Figs. 1—8.—These are drawings made from the central nervous system of Lemur 9, in which the face region was removed. The position of the lesion is indicated by a dotted line in fig. 1, which is a drawing of a section through the injured (right) hemisphere. The preparations were made by the Marchi method, and degeneration is indicated by black lines and dots. Fig. 1 shows degeneration passing by the corpus callosum to the opposite hemisphere, by association tracts to adjacent convolutions, and by the internal capsule to the cerebral peduncle.

Fig. 2 is a drawing of a section through the left (uninjured) hemisphere, and shows the degenerated fibres, reaching the grey matter, which have crossed by the corpus callosum. Some few degenerated fibres are seen in the internal capsule.

Fig. 3 is the mid-brain. Degeneration is seen in the crusta of the peduncle, especially in its outer and inner fourths.

Fig. 4 shows the degenerated fibres in the pyramidal tract of the pons.

Figs. 5 and 6 show them in the pyramidal tract of the bulb. In figs. 4, 5, and 6 many of the degenerated fibres are seen decussating to reach the motor nuclei of cranial nerves.

Fig. 7, through the upper cervical region of the spinal cord, shows a few degenerated fibres in the crossed pyramidal tract of the opposite side.

Fig. 8, through the cervical enlargement, shows that at this level all degenerated fibres have disappeared.

Figs. 9—16.—A similar series of drawings made from the central nervous system of Lemur 8, in which a large portion of the lower limb area was removed.

Fig. 9 is a section through the injured (right) hemisphere.

Figs. 10, 11, 12, and 13 show the degenerated fibres in the pyramidal tract at various levels in pons and bulb. Decussation of these does not begin until a lower level is reached than in the case of Lemur 7 (removal of arm area, see Plate 2).

Figs. 14, 15, and 16 are drawings of the spinal cord—14 at the level of the 8th cervical and 15 at the level of the 6th dorsal segment. In fig. 15 the degenerated fibres are nearly as numerous as in fig. 14, and also nearly as numerous as in the pyramidal tract of the bulb. It is not until the lumbar region is reached that they begin to diminish (fig. 16, taken at 5th lumbar level).

Plate 4.

This shows the two types of motor cortex described in the text. Fig. 1 is the first type of motor cortex, characterised by giant pyramids (Betz cells), and found in the limb and trunk areas. Fig. 2 is the second type of motor cortex with smaller Betz cells, found in the face, ear, and eye regions. Higher power views of typical cells from each layer are placed by the side of the low power drawings. Methylene blue preparations.

On the Structure of Sigillaria scutellata, *Brongn., and other Eusigillarian Stems, in Comparison with those of other Palæozoic Lycopods.*

By E. A. Newell Arber, M.A., F.L.S., F.G.S., Trinity College, Cambridge University Demonstrator in Palæobotany, and Hugh H. Thomas, B.A., formerly Scholar of Downing College, Cambridge.

(Communicated by D. H. Scott, F.R.S. Received November 8,—Read December 5, 1907.)

(Abstract.)

Petrified stems belonging to the genus *Sigillaria* have hitherto proved to be extremely rare in the Upper Carboniferous rocks. The present paper contains the first full account of the structure of the Eusigillariæ or ribbed Sigillarias of the Rhytidolepis section. Hitherto our knowledge of the anatomy of such stems has been limited to the account of the bark given by Williamson, and to brief descriptions of specimens, including the vascular cylinder, by Professor Bertrand and Dr. Scott.

The material, which forms the basis of the present study, consists of a petrifaction from the Lower Coal Measures of Shore-Littleborough in Lancashire, containing two well-preserved stems, lying side by side. The external surfaces of the ribs of both stems have been exposed by Mr. James Lomax, after much difficulty, and our thanks are due to him for his skill in preparing the sections. The characters of the ribs agree with those of the impressions known as *Sigillaria scutellata*, Brongn.

In addition, other stems are described in which the ribs are not exposed, and which cannot, therefore, be determined specifically. Radial and tangential sections through the bark of all these specimens show, however, that they belong to species of the Rhytidolepis section of the Eusigillariæ, which, like *Sigillaria scutellata,* possessed distant leaf scars. These additional petrifactions agree exactly with *Sigillaria scutellata,* and have been made use of to illustrate further the anatomy of that species.

The stele has a well-marked pith, a tissue which is not, however, preserved in any of the stems which we have examined. The medullary cavity is bounded by a continuous ring of scalariform tracheides,—the primary wood,—the outer margin of which is crenulated. The protoxylem elements lie at the apices of the blunt, rounded teeth of the corona. The elements of the protoxylem and primary wood appear to consist entirely of scalariform tracheides. The elements of the secondary wood are also scalariform, and

rather smaller than those of the primary xylem, but, unlike them, they are arranged radially. The outer margin of this zone was crenulated, the ridges and grooves corresponding in position to those of the primary wood. The medullary rays usually consist of a single row of cells of varying height, of which the walls are sometimes thickened transversely.

The phloem and inner cortex of thin-walled elements are not preserved. A well developed band of phelloderm is found near the surface of the ribs. This is regarded as having arisen on the inner side of a meristematic zone. No definite cambial layer is to be found, and it is suggested that the meristematic activity here took place periodically. Cells are to be seen in this region which appear to have undergone division shortly before preservation took place, and rings of growth are to be observed in the older portions of the phelloderm. The secondary tissue consists of prismatic fibres, often chambered.

The ribs are really formed of cortical tissues, and not by fused leaf-bases. They consist largely of phelloderm, and externally what is probably a small zone of primary cortex, which lay without the region of secondary meristermatic activity, still persists. The stems were probably ribbed long before the formation of the periderm. The leaf-bases, consisting of thin-walled parenchymatous elements, merely form bracket-like projections from the ribs; those of the same vertical series being sufficiently distant from each other to leave a small gap of primary cortex between them. The ribbing of the stem in the Eusigillariæ, being entirely independent of the form and arrangement of the leaf-bases, appears to be a natural feature of importance in classifying the Sigillariæ. No sign of branching has been observed in any specimen. The presence of a ligule and a ligular pit has been detected for the first time.

The course of the leaf-traces in the leaf-bases and cortical tissues has been followed with important results. The bundle is collateral, and without secondary wood. In the leaf-bases the trace consists of a *double xylem strand,* the two xylem groups being widely separated. These two strands unite as they pass through the phelloderm. The structure of the trace is almost identical with the foliar bundle of the leaf described by Scott as *Sigillariopsis sulcata,* which is obviously simply the leaf of a Eusigillarian stem.

The parichnos—the two strands of thin-walled elements, which accompany the leaf-trace through the leaf-base and cortex—increases greatly in size, as we pass from the exterior of the stem to the inner margin of the periderm. The two strands further unite, first below and then above the trace, so that, at a deep level in the periderm, the trace is completely surrounded by a broad zone of this tissue.

The leaf-traces pass through the secondary wood at first at an angle of about 60° to the vertical, but their course soon becomes almost horizontal, and this is maintained until near the inner margin of the wood, when they again bend sharply downwards, and eventually unite with the primary wood in one of the grooves of the corona.

The Eusigillariæ are compared anatomically with the Subsigillariæ, and it is found that there are four points in which they differ. In the Eusigillariæ, the stems are ribbed and the primary xylem always forms a continuous ring. The leaf-traces are monoxylic throughout their course. In the periderm, the xylem of the trace divides into two distinct strands, and these persist through the leaf-base, into the leaf, until near its apex, as the xylem of the foliar bundle. If, however, we regard *Sigillariopsis Decaisnei*, Ren., as a member of the Subsigillariæ, a conclusion which seems inevitable, then this latter characteristic is common to both groups, though in the Subsigillariæ it is combined with the diploxylic structure.

The Eusigillariæ are next compared with the various types of structure exhibited by *Lepidodendron* and *Lepidophloios*, with the conclusion that they correspond most closely to the Lepidodendroid trunks of Arran and Dalmeny. Anatomically they appear to be remote from *Bothrodendron*, so far as the structure of that genus is known. It is found that, in the absence of the cortical tissues, it is not possible to distinguish the stele of a Eusigillarian stem by any definite characters from that of some Lepidodendreæ. The genus *Diploxylon* is discussed in this connection, and it is shown that it is by no means certain that all the decorticated stems, which have been referred to it, belong to the Sigillariæ. It is more probable that the stems of several distinct genera are here grouped together, if only as a temporary expedient.

*Dietetics in Tuberculosis: Principles and Economics.**

By Noel Dean Bardswell, M.D., M.R.C.P., F.R.S. (Edin.), Medical Superintendent, King Edward VII Sanatorium, and John Ellis Chapman, M.R.C.S., L.R.C.P., Medical Superintendent, Coppin's Green Sanatorium.

(Communicated by Sir T. Clifford Allbutt, K.C.B., F.R.S. Received November 26, 1907,—Read January 23, 1908.)

Object of Research.—The object of our research was to obtain reliable data upon which to draw conclusions as to the best lines upon which to base the dietetic treatment of pulmonary tuberculosis. In 1899, when this work was first commenced, the sanatorium treatment of consumption and other forms of tuberculosis was rapidly becoming adopted in this country. One of the most noteworthy features of this treatment was the systematic prescription of diets of a very high nutritive value. In the absence of any reliable authorities on the dietetics of tuberculosis, the practice of giving very large diets became very general, in spite of warnings from physiologists that such a method of treatment was probably unsound. Some preliminary observations, which we made at Sheffield Royal Infirmary in 1899 upon the metabolism of several consumptive patients treated on very large diets, suggested to us that an extended series of such observations might enable us to place the dieting of tuberculosis upon a more scientific foundation. Our series of observations has extended over seven years, and this paper represents an abstract of our final report.

Research 1.

We hoped, as a result of our first series of observations, to establish :—

(1) The best general principles upon which to construct diets for the treatment of tuberculosis.

(2) A standard diet in terms of proteid, fat, carbohydrate, and total calorie value for the treatment of tuberculosis.

Method of Observation.—The progress made by 200 cases of pulmonary tuberculosis, representing well-marked stages of the diseases, were carefully observed whilst treated on definite diets, the general lines of treatment being the same in every case.

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* Towards the expenses of this research, the authors, on the recommendation of the Royal Society, were given a Government Grant. This paper is a Summary of the Final Report; the full Report will be published shortly by the Oxford University Press.

In the case of every patient that was observed, we first determined the diet which was physiological for the individual, when in ordinary health, up to his average body weight, and at physiological rest. This physiological diet was then increased in certain definite amounts of proteid, fat, or carbohydrate. The actual diet prescribed was carefully constructed so as to give the nutritive value decided upon. This diet was given in measured and weighed amounts, and anything left was also weighed or measured. In this way an accurate record of the food actually consumed by the patient was arrived at and its nutritive value calculated. Careful clinical observations were made on all the patients, especially as to the improvement in the morbid process in the lungs, gain of weight, and improvement in general health. In a large proportion of cases, metabolic observations were also made. Sometimes these observations were made during a four-day period once a month; in other cases they were made daily for periods varying from a week to three months. The points especially studied were :—

(1) The absorption of fat and nitrogen.

(2) The amount of the excretion of nitrogen.

(3) The form in which the nitrogen was excreted, viz., whether simple or in the more highly elaborated forms, and their percentage relation.

(4) The amount of intestinal putrefaction, as evidenced by the ratio between the aromatic and the alkaline sulphates excreted in the urine.

Conclusions of Research 1.—The following are satisfactory principles upon which to construct dietaries for tuberculous patients :—

(1) The physiological diet (viz., the diet which contains the exact amount of carbon and nitrogen necessary to balance the amounts of the substances excreted) for every tubercular individual when in normal health and at physiological rest, should first be ascertained, and this physiological diet should form the basis of the diet prescribed for the treatment.

(2) The amount of proteid in the physiological diet should be increased by 30 per cent., and this increase should be maintained until the disease is obsolete.

(3) If the patient is much under weight, the calorie value of the physiological diet should also be increased 30 per cent. in the purely energy-giving foods, viz., in fats or carbohydrates, or in both. This increase should be maintained until the weight becomes stationary, at a point a few pounds in excess of the patient's normal weight. A decrease of 15 per cent. can then be made, and the diet thus altered should be continued until the disease is obsolete.

(4) The meals must not be too bulky, but somewhat inclined to concentration, so as to give the comparatively large amount of nourishment in a but

slightly increased bulk of food stuffs. In the case of the consumptive working classes this last rule does not always apply, since they are used to taking diets of large bulk.

(5) The meals should be given at considerable intervals; they should be well cooked and as varied as possible.

Standard Diet for the Treatment of the average Tubercular Patient.

The following table gives the nutritive value of the average diets taken by 49 tubercular patients throughout their course of sanatorium treatment. These 49 patients have been selected inasmuch as they all made very good recoveries.

The average of these 49 satisfactory diets works out at

Proteid.	Fat.	Carbohydrate.	Calories.
150	150	250	3000

These figures, in our opinion, may be taken as representing the nutritive value of a diet which is satisfactory as a standard diet for the treatment of the average person suffering from tuberculosis. We have, as a matter of fact, adopted this standard in our sanatorium practice for the past few years, and found it to be most satisfactory. The standard diet which we have found to be best for tubercular women has a somewhat lower nutritive value, viz.:—

Proteid.	Fat.	Carbohydrate.	Calories.
126	150	220	2814

The following actual diets give the nutritive value of the above standard diets:—

Article of food as served.	Amount prescribed.	
	For men.	For women.
Milk	1500 c.c.	1500 c.c.
Bread	180 grammes.	135 grammes.
Porridge	120 "	120 "
Butter	45 "	45 "
Breakfast meat, such as bacon, fish, etc.	30 "	30 "
Ordinary meat	180 "	150 "
Pudding	300 "	180 "
Vegetables	q.s.	q.s.

Table I.

	On admission.					Diet.			On discharge.			
	No. of lobes diseased.	Temperature range, ° C.	Digestion.	Weight in kilos.	Relation to normal weight.	Proteid.	Fat.	Carbo-hydrate.	Lung disease.	General health.	Weight in kilos.	Temperature range, ° C.
Cases with early disease.	3	37·9—38·4	Normal	57·0	− 6·3	161	154	255	Complete arrest	Complete restoration	67·8	Normal
	2	Normal	Poor	57·0	− 5·4	152	121	226	Incomplete arrest	Excellent	64·5	,,
	2	37·2—37·9	Normal	66·9	− 5·8	168	162	278	Complete arrest	Complete restoration	71·8	,,
	1	36·9—37·6	,,	76·0	equal to	172	156	268	,, ,,	,, ,,	76·9	,,
	2	Normal	Impaired	58·0	− 6·3	184	166	268	Incomplete arrest	,, ,,	62·0	,,
	2	37·9—38·4	Normal	57·0	− 6·8	145	138	247	,, ,,	,, ,,	71·0	,,
	1	36·6—37·5	,,	57·5	− 3·0	136	132	228	Complete arrest	,, ,,	63·8	,,
	1	36·6—37·9	,,	51·1	− 9·9	155	148	260	Incomplete arrest	Largely restored	57·0	,,
	3	37·0—37·6	,,	53·0	− 7·7	160	156	270	Complete arrest	Complete restoration	65·2	,,
	1	Normal	,,	76·1	− 5·8	162	158	242	,, ,,	,, ,,	82·3	,,
	2	37·2—37·9	,,	63·0	−11·7	153	148	256	,, ,,	,, ,,	76·9	,,
	1	37·2—37·6	,,	58·0	− 7·7	151	153	242	,, ,,	,, ,,	64·4	,,
	2	37·9—38·4	,,	56·5	− 3·0	168	155	282	,, ,,	,, ,,	64·4	,,
	2	37·0—37·5	,,	61·1	− 9·4	180	156	276	,, ,,	,, ,,	68·5	,,
	2	Normal	,,	64·7	− 5·8	153	152	232	,, ,,	,, ,,	67·3	,,
	1	,,	,,	59·5	equal to	145	150	254	,, ,,	,, ,,	64·2	,,
	1	,,	,,	52·5	− 6·3	151	144	248	,, ,,	,, ,,	63·7	,,
	1	,,	,,	60·0	− 6·3	148	150	246	,, ,,	,, ,,	69·6	,,
	1	,,	,,	72·8	equal to	151	150	248	,, ,,	,, ,,	75·0	,,
	1	,,	,,	56·2	equal to	152	148	256	,, ,,	,, ,,	63·6	,,
	2	,,	,,	60·9	− 2·2	156	150	252	Incomplete arrest	,, ,,	65·8	,,
	1	,,	,,	69·6	equal to	162	178	236	Complete arrest	,, ,,	71·8	,,
	2	,,	,,	68·3	− 1·5	171	156	256	,, ,,	,, ,,	76·0	,,
	1	,,	,,	63·0	− 2·4	158	158	264	,, ,,	,, ,,	64·2	,,
	2	,,	,,	63·6	− 2·8	160	152	272	,, ,,	,, ,,	71·0	,,
	1	,,	,,	70·5	equal to	162	150	282	,, ,,	,, ,,	74·6	,,
	1	,,	,,	60·0	equal to	152	133	198	,, ,,	,, ,,	61·0	,,
	3	,,	,,	87·2	− 2·8	138	120	280	,, ,,	,, ,,	87·8	,,
Cases with advanced disease.	4	37·2—38·0	Normal	58·8	−12·6	143	181	255	Incomplete arrest	Incomplete restoration	67·0	37·2—37·8
	5	36·7—37·4	,,	51·5	− 5·4	120	148	239	Very considerably arrested	Excellent	58·8	36·9—37·4
	4	37·2—38·3	,,	45·7	−13·1	155	135	235	,, ,,	Very greatly improved	56·5	Normal
	2	Normal	,,	76·0	equal to	148	142	245	,, ,,	,, ,,	76·0	,,
	5	37·1—37·4	Impaired	61·5	− 4·5	160	150	244	Complete arrest	Complete restoration	70·0	,,
	3	37·0—37·3	Very fair	49·7	− 6·3	147	155	261	Incomplete arrest	Very good	55·6	37·0—37·5
	4	Normal	,,	65·6	− 6·3	151	147	252	,, ,,	Complete restoration	70·0	Normal
	3	37·0—37·4	Poor	54·0	− 6·3	158	136	220	Complete arrest	,, ,,	60·0	,,
	3	36·9—38·9	Very fair	62·0	− 8·10	162	155	271	Incomplete arrest	Perfectly restored	76·0	36·9—37·3
	3	Normal	Normal	73·3	− 8·10	161	153	266	,, ,,	Excellent	85·0	36·9—37·3
	2	37·2—37·4	,,	52·4	− 3·1	158	160	260	,, ,,	,,	61·0	36·9—37·3
	3	37·1—37·4	,,	55·6	− 4·0	134	134	188	Very much improved	Very much improved	57·9	Normal
	3	36·9—37·5	,,	55·7	− 5·0	150	136	240	Much improved	Largely restored	62·4	,,
	3	Normal	,,	66·0	− 2·7	153	138	248	Very much improved	,, ,,	62·4	,,
	3	36·9—37·5	Impaired	62·0	− 5·9	140	142	250	,, ,,	,, ,,	69·2	,,
	3	36·9—37·5	,,	49·3	−16·3	153	158	275	Very largely arrested	,, ,,	59·3	,,
	2	Normal	,,	61·0	− 2·7	148	148	278	,, ,,	,, ,,	63·8	,,
	3	37·0—37·5	Good	70·6	equal to	148	142	242	Very much improved	Much improved	71·0	,,
	4	Normal	Impaired	55·2	− 4·5	151	161	273	Very considerably arrested	Very much improved	60·0	,,
	1	37·2—38·0	Poor	71·0	−11·0	141	138	231	Much improved	Good	73·7	37·1—37·5
	5	37·2—37·5	,,	66·0	− 2·7	169	145	198	Improved	Improved	67·0	Normal

Definition of Terms: *Complete Arrest.* No signs in lungs incompatible with a completely healed lesion.

Incomplete Arrest. Signs of disease, though much less marked, not entirely cleared up.

Observations on Tubercular Patients treated with Very Large Diets.

Careful observations have been made upon tubercular patients treated on the very large diets prescribed in many sanatoria, for instance, on diets with a daily proteid value of 200 grammes or more, and a total calorie value of 4000 or more.

Clinical Results of these Observations.—Patients made much less satisfactory all round progress on the very large diets than on diets of considerably smaller nutritive value. Weight was gained in nearly every case, in some to a very large extent, and very rapidly, but this gain of body-weight was not associated with any more satisfactory progress in the tubercular lesions than was obtained with the smaller diets; on the other hand, general health suffered considerably, as evidenced by failure of appetite and marked digestive and intestinal derangements.

Metabolism.—(1) The absorption of fat in the case of patients below their normal weight was higher than normal, even when very large quantities were ingested, *e.g.*, a patient taking 231 grammes of fat daily absorbed 96·4 per cent. The great majority of patients absorbed over 90 per cent., and in no case was the absorption below 87 per cent.

(2) The absorption of nitrogen was also high in all cases, viz., 90 per cent., or over, but, when very large quantities were ingested, an increase in the amount excreted in the urine invariably occurred, and only a very small percentage of the increased amount of nitrogen ingested remained in the body. It was noticeable that the absorption, both of fat and nitrogen, was high, even in the cases suffering from acute dyspepsia.

(3) The percentage of nitrogen excreted as urea decreased, and, consequently, the percentage excreted in less oxidised form increased, indicating less complete elaboration.

Table II.—Diets and Metabolism of Three Patients treated by Forced Feeding.

Proteid	232·5	271·1	251·4	Nutritive value of diet.
Fat	183·9	231·2	208·3	
Carbohydrate	321·3	392·1	297·2	
Total calories	4126·0	5026·0	4187·0	
Fluids	2810·0	3444·0	3234·0	
N in urine	30·8	23·8	18·1	
Urea	35·5	40·6	28·4	
N in fæces	2·1	4·3	2·4	Metabolism figures (average of 4 days' observation).
Fat	3·8	7·8	4·4	
N absorbed (per cent.)	94·2	89·9	93·9	
Fat (per cent.)	97·9	96·4	97·7	

(4) The ratio of the aromatic to the alkaline sulphates excreted in the urine became smaller, indicating increased intestinal putrefaction.

The preceding table shows the nutritive values of the diets taken by three patients thus treated by forced feeding, and the results of metabolic observations during a four-day period.

Research 2. On the most Economical Lines upon which to Construct Diets having the same Nutritive Value as our Standard Diet.

In view of the large expense of dieting in most sanatoria, and in order to bring the modern dietetic treatment of tuberculosis within the reach of the poorer classes, we made a series of observations, with a view to determining the most economical lines upon which an adequate diet can be constructed.

An Economical Diet.—An analysis we made of physiologically adequate diets, taken by 100 working-class families, showed us that an adequate diet for the working classes can be bought for about 10*d.* a day, and that, in such a diet, every penny spent buys some 12·7 grammes of proteid and 329 calories. The average of these 100 satisfactory diets worked out at: proteid, 119; fat, 114; carbohydrate, 417; calories, 3687; at a cost of 10*d.* (not including money spent upon beer or other alcoholic drinks).

The aim of our work was to increase this average diet some 30 per cent. in proteid without appreciably increasing the cost.

During the course of our preliminary observations on the subject, we found that the convalescent working-class consumptive, when taking a considerable amount of exercise, such as digging in the gardens, etc., requires a larger diet than our standard diet for men at physiological rest, and we aimed, in consequence, at constructing a diet with a nutritive value of

Proteid.		Calories.
150	and	3600 approximately.

A trial of several dietaries of the above nutritive value, and constructed on the lines of ordinary dietaries, showed us that the expenditure on meat accounted for some 40 per cent. of their total cost, and that, to construct a really cheap diet, the amount of animal proteid, especially meat, must be kept down as much as possible, and considerable use made of the cheaper forms of vegetable proteid, such as peas, beans, lentils, etc. We estimated that the replacing of meat by vegetable proteid would prove an economy of 30 per cent.

In view of the widely accepted principle that animal proteid, especially meat, has some specific value in the treatment of tuberculosis, and may even be regarded as an essential in the diets for the tubercular, we decided first to

Table III.

Case.	No. of days under observation.	Nutritive value of diet.				Percentage of animal proteid.	Percentage of vegetable proteid.	Cost, in pence.	Weight of food taken daily, in grms.	Weekly gain in weight, in kilos.	Total gain in weight, in kilos.
		Proteid.	Fat.	Carbohydrate.	Calories.						
		Cases of Early Disease with Normal Digestions.									
1	71	175·2	146·8	550·5	4340	44·0	56·0	11·25	4331	1·5	13·5
2	55	156·1	149·6	501·4	4085	46·1	53·9	10·81	4176	1·5	8·3
3	63	164·9	125·0	456·5	3710	48·3	51·7	10·86	3527	0·9	9·0
		Cases of Advanced Disease with Impaired Digestions.									
4	47	118·7	99·4	289·0	2596	49·2	50·8	7·39	3047	Stationary	Nil
5	72	125·8	110·8	366·5	3049	43·9	56·1	8·11	3160	0·3	2·25
6	21	120·9	96·0	335·0	2761	48·0	52·0	6·26	3111	1·75	2·2
Average	55	143·6	121·3	416·5	3473	46·6	53·4	9·11	3558	0·99	5·7

Table IV.—Meat-free Diet taken by Case 2 for 55 days.

——	Amount.		Nutritive value.			Cost, in pence.
	Grammes.	Ounces.	Proteid.	Fat.	Carbohydrates.	
Whole milk	2000	70	66·0	80·0	100·0	5·28
Meat	Nil	—	—	—	—	—
Butter	71	2½	—	56·8	—	1·86
Cheese	—	—	—	—	—	—
Eggs	1	1	6·0	4·0	—	0·66
Bacon	Nil	—	—	—	—	—
Total animal food	—	—	72·0	140·8	100·0	7·80
Bread	268	9	23·8	2·6	132·5	0·72
Potatoes	—	—	—	—	—	—
Oatmeal	50	2	8·0	3·6	33·7	0·22
Peas, beans, etc.	200	7	48·6	2·6	120·6	0·87
Sugar	40	1½	—	—	40·0	0·22
Rice, etc.	28	1	2·2	—	22·4	0·12
Jam, etc.	59	2	0·5	—	47·2	0·46
Green vegetables	q.s.	—	1·0	—	5·0	0·10
Sundries	—	—	—	—	—	0·30 (entirely unnutritive)
Total vegetable food	—	—	84·1	8·8	401·4	3·01
Total food	—	—	156·1	149·6	501·4	10·81

Calories bought per penny, 378. Grammes of proteid bought per penny, 14·8.
Total calorie value, 4085.

satisfy ourselves as to the truth of the principle. For this purpose, we carefully observed six typical cases of pulmonary tuberculosis treated on an entirely meat-free diet. A summary of the results of these observations is shown in the following table, and a sample diet taken by one of these patients for a period of 55 days is also given.

Results of Observations as to the Value of a Meat-free Diet for the Treatment of Tuberculosis.

Our conclusions are as follows :—

(1) Vegetable proteid, as the main source of the daily intake of proteid in a diet for the tubercular, is thoroughly satisfactory, so long as a sufficient amount of it is taken.

(2) The clinical results obtained, when treating consumptives with good digestions upon meat-free diets of an adequate nutritive value, are quite as good as the results that are obtained when ordinary meat diets of a similar nutritive value are used.

(3) Owing to the bulky nature of a meat-free diet, its use is restricted to patients with normal appetites and digestions; it is unsuitable for the treatment of those with marked impairment of the alimentary tract.

(4) The use of vegetable proteid in the place of all the meat usually prescribed in an ordinary meat diet effects an economy of some 33 per cent.

On the Construction of a Cheap Diet containing an Ordinary Amount of Animal Proteid, such as Meat, etc., to Ensure Palatability and Variety, and a Certain Amount of Vegetable Proteid for the Purpose of Economy.

Our practical experience with meat-free diets showed us that, in spite of their great economy, they are not quite satisfactory, inasmuch as they require very careful cooking to make them appetising, and that even when well cooked they are not readily taken by the ordinary person accustomed to a meat dietary. To be really efficient, a diet must be to the liking of those to whom it is prescribed.

We then constructed several dietaries in which the 150 grammes of proteid which we consider to be desirable in the treatment of tuberculosis is given, partly in the form of meat and partly in the form of vegetables. The following table shows one of these diets which was taken by three tubercular patients for an average period of 26 days :—

Table V.—Diet taken by Three Men during an average period of 26 days.

—	Amount.		Price retail.	Nutritive value.			Cost, in pence.
	Ounces.	Grammes.		Proteid.	Fat.	Carbo-hydrate.	
Milk (separated)	42	1200	1*d.* per pt.	40	3	60	2·11
Meat*	7	200	7*d.* per lb.	36	24	—	3·16
Suet	½	16	6*d.* ,,	—	13	—	0·18
Margarine	2	54	8*d.* ,,	—	43	—	1·00
Cheese (American)	⅓	10	7*d.* ,,	3	4	—	0·15
Egg	—	—	—	—	—	—	—
Bacon	2	56	6*d.* per lb.	4	19	—	0·75
Total animal food	—	—	—	83	106	60	7·35
Bread	10	282	2½*d.* per 2 lbs.	26	3	141	0·78
Potatoes	7½	210	8*d.* per stone	2	—	30	0·26
Peas, beans, etc.	3¼	97	2*d.* per lb.	21	1	57	0·40
Oatmeal	2	56	2*d.* ,,	8	4	37	0·25
Sugar	1½	40	2½*d.* ,,	—	—	40	0·22
Jam	2	56	3½*d.* ,,	—	—	33	0·44
Rice, etc.	½	16	2½*d.* ,,	1	—	13	0·08
Flour	2	56	1*s.* 6*d.* per stone	7	1	39	0·16
Green vegetables	6	170	—	1	—	8	0·25
Sundries	—	—	—	4	—	10	0·50
Total vegetable food	—	—	—	70	9	408	3·34
Total food	—	—	—	153	115	468	10·69

Total calorie value, 3616. Ratio of cost of animal to vegetable food, 68·7 : 31·3.
Grammes of proteid per penny, 14·3. Calories per penny, 337.

* Uncooked and including bone, etc., and comprising beef, mutton, pork, tinned beef, mutton, etc.

The above diet could be bought retail in London from 6*s.* to 6*s.* 6*d.* per head per week for an average sized family, according to the quality of food purchased.

The results obtained in the three cases observed on this diet were most satisfactory.

After very considerable further experience in the treatment of tuberculosis with cheap dietaries constructed on the lines which we have described, we have adopted the following dietary as being, in every way, the most efficient and satisfactory:—

Table VI.—Diet taken by 15 Patients at Coppin's Green Sanatorium during the week of observation, per man per diem.

—	Ounces.	Grammes.	Proteid.	Fat.	Carbo-hydrate.	Price per lb. or gallon.	Cost, in pence.
Milk	24·4	690	23·0	28·0	34	1*s.*	1·87
Meat	8·65	245	44·0	28·0	—	6½*d.*	3·00
Liver, etc., fish	2·0	56	10·0	7·0	—	5*d.*	0·63
Cheese	0·5	14	5·3	1·4	—	6*d.*	0·19
Dripping	0·46	13	—	11·2	—	—	—
Butter	1·05	29	—	23·0	—	1*s.*	0·68
Egg (1 per week)	1/7	—	1·0	0·7	—	—	0·14
Bacon	2·0	56	8·0	17·0	—	7*d.*	0·87
Total animal food	—	—	91·3	116·3	34	—	7·38
Bread	8·7	247	22·0	2·0	123	1¼*d.*	0·69
Potatoes	8·0	228	3·0	—	30	6*d.* per 14 lbs.	0·23
Pulse	3·0	85	20·0	—	53	2*d.*	0·38
Oatmeal	2·0	56	9·0	4·0	38	1½*d.*	0·19
Sugar	5·3	150	—	—	150	2*d.*	0·66
Jam	1·0	28	—	—	21	3*d.*	0·19
Cereals	0·36	10	—	—	7	2½*d.*	0·05
Flour	2·0	56	6·0	—	39	1*s.* 6*d.* per stone	0·15
Sundries	q.s.	—	2·0	3·0	16	—	0·60
Total vegetable food	—	—	62·0	9·0	477	—	3·14
Total food	—	—	153·3	125·3	511	—	10·52*

Grammes of proteid bought per penny, 13·2. Calories per penny, 336. Total calorie value, 3889.

* True cost, allowing 10 per cent. for waste in cooking as explained = 11·57*d.*

This dietary has a thoroughly adequate nutritive value for the treatment of tuberculosis. With a daily value of proteid, 154 grammes and 3889 calories, it is especially suitable for consumptives who are convalescent and doing a certain amount of muscular work.

It is very palatable, easily digested, and allows of a considerable variety being made in the menu day by day.

It is very cheap, considering the amount of nourishment which it contains, costing only 11½*d.* per day.

It is very economically constructed, every penny spent upon it buying 32 grammes of proteid and 336 calories.

On the Weight of Precipitum obtainable in Precipitin Interactions with Small Weights of Homologous Protein.

By Professor D. A. Welsh and Dr. H. G. Chapman.

(Communicated by Dr. C. J. Martin, F.R.S. Received December 17, 1907,—Read February 6, 1908.)

(From the Physiological and Pathological Laboratories of the University of Sydney.)

In a previous communication* on precipitin reactions, we brought forward observations which led to the conclusion that the precipitum is derived mainly from the antiserum and not from the homologous protein. In a recent review† of our paper the suggestion was made that gravimetric evidence might be more conclusive. Acting on this suggestion, we have carried out experiments in which a considerable amount of antiserum was allowed to interact with a known small amount of homologous protein. As soon as the interaction was completed, the deposits were collected, washed, and weighed. In every case the amount of dried precipitum exceeded the amount of dried homologous protein. The smallest precipitum obtained weighed more than twice, the largest more than 25 times, the homologous protein employed in the interaction. Former observations‡ had revealed that antisera require very different amounts of protein to ensure the formation of maximal deposits. It was, therefore, to be expected that considerable variations would be obtained in the weight of deposits produced by small amounts of protein in different antisera.

Method of Experiment.

The antiserum was obtained from rabbits which were bled on the day of the experiment. One milligramme or 2·5 milligrammes dried egg-white or blood serum was dissolved in 10 c.c. salt solution and added to the homologous antiserum, together with about 90 c.c. salt solution. The whole of the available clear antiserum, with the exception of less than 1 c.c. reserved for control observations, was employed. The dried protein was prepared under aseptic conditions, and every precaution was taken to exclude micro-organisms§

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* Welsh and Chapman, 'Roy. Soc. Proc.,' B, vol. 78, p. 297, 1906.

† Mouton, 'Bulletin de l'Institut Pasteur,' 1907.

‡ Welsh and Chapman, 'Journ. of Hygiene,' vol. 6, p. 263, 1906.

§ We are unable to confirm Friedberger, 'Centralb. f. Bakt., Orig.,' vol. 43, pp. 490—494, March 5, 1907, in his statements regarding the prevention of bacterial growth by precipitin antisera, as our tubes tend to become infected in about 7 days.

during the experiment. After 48 hours the clear superfluids were removed, and were usually subjected to further experiment, either by addition of more homologous protein, or by addition of fresh antiserum from another immunised animal. Since it had been shown* that different homologous antisera might interact to yield a precipitate, appropriate controls were carried out which showed that no trace of deposit occurred as the result of the interactions with one another of the antisera used in these experiments.

The deposits from the interactions were shaken up three times with 50 c.c. saline solution, and separated by the centrifuge. They were then washed five times with 50 c.c. distilled water, whereupon the superfluid yielded no trace of soluble protein. After three washings with absolute ethyl alcohol, they were finally treated with ether free from water. On removal of the excess of ether with a pipette, the deposits were placed in an oven at 50° C. for 24 hours, and dried to constant weight in a desiccator over calcium chloride. The weighings were made in small glass test-tubes of about 7 grammes tare, in which the final washings with alcohol and with ether had been carried out.

Amount of Precipitum obtainable from Large Quantities of Antiserum with Small Amounts of Homologous Protein.

Five experiments were undertaken, and the results are summarised in Table I.

Table I.

No. of experiment.	Weight of homologous protein (dried).	Amount of antiserum (fresh).	Weight of precipitum (dried).
			milligrammes.
1	2·5 milligrammes hen egg-white......	19 c.c. hen egg antiserum (No. 40) ...	18·0
2	2·5 milligrammes hen egg-white......	20 c.c. hen egg antiserum (No. 41) ...	18·0
3 (*a*)	1·0 milligramme horse serum	19 c.c. horse antiserum (No. 43)	2·2
3 (*b*)	Superfluid of 3 (*a*)	13 c.c. horse antiserum (No. 42)	5·0
4 (*a*)	1·0 milligramme ostrich egg-white...	20 c.c. ostrich egg antiserum (No. 44)	5·0
4 (*b*)	4·0 milligrammes ostrich egg-white	Superfluid of 4 (*a*)	11·0
5 (*a*)	1·0 milligramme hen egg-white	18 c.c. hen egg antiserum (No. 47) ...	13·0
5 (*b*)	Superfluid of 5 (*a*)	16 c.c. hen egg antiserum (No. 46) ...	8·5
5 (*c*)	Superfluid of 5 (*b*)	18 c.c. hen egg antiserum (No. 48) ...	4·4

In experiment (1) 2·5 milligrammes dried hen egg-white interacted once with 19 c.c. fresh hen egg antiserum (No. 40) and caused the formation of a deposit weighing 18 milligrammes, or more than seven times the weight of the homologous protein concerned.

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* Nuttall, Hopkins, and Strangeways, quoted by Nuttall, 'Blood Immunity and Blood Relationship,' p. 129, Cambridge, 1904.

In experiment (2) also 2·5 milligrammes dried hen egg-white interacted once with 20 c.c. fresh hen egg antiserum (No. 41), and yielded a precipitum of 18 milligrammes.

In experiment (3) 1 milligramme dried horse blood serum, interacting (*a*) with 19 c.c. fresh horse antiserum (No. 43) produced a deposit of 2·2 milligrammes, or more than twice the weight of the homologous protein. The residual homologous protein in the clear superfluid, interacting (*b*) with 13 c.c. of a second fresh horse antiserum (No. 42), yielded a further deposit of 5 milligrammes, making 7·2 milligrammes in all, or more than seven times the amount of the original homologous protein.

In experiment (4) (*a*) 1 milligramme dried ostrich egg-white, interacting with 20 c.c. fresh ostrich egg antiserum (No. 44), led to a deposit of 5 milligrammes, and (*b*) the subsequent addition of 4 milligrammes dried ostrich egg-white to the clear superfluid led to a further deposit of 11 milligrammes.

In experiment (5) 1 milligramme dried hen egg-white, interacting (*a*) with 18 c.c. fresh hen egg antiserum (No. 47), brought down a deposit of 13 milligrammes. The residual protein in the clear superfluid, interacting (*b*) with 16 c.c. of a second fresh hen egg antiserum (No. 46), gave a further deposit of 8·5 milligrammes. The resulting superfluid, again interacting (*c*) with 18 c.c. of a third hen egg antiserum (No. 48), gave a third deposit of 4·4 milligrammes. Thus, by allowing 1 milligramme of the homologous protein to interact successively with three antisera (which did not mutually interact), a total deposit of 25·9 milligrammes was attained.

The progressively diminishing weights of precipitum obtained from the successive antisera in experiment (5) (*a*), (*b*), and (*c*), do not necessarily indicate that the homologous protein is being appreciably used up in the interactions. A difference in weight of deposit may be an indication merely of a difference in precipitability of antiserum, a circumstance to which we have already referred. This interpretation is consistent with the control observations made with each of these antisera and fresh homologous protein, and also consistent with the progressively increasing weights of deposit obtained in similar circumstances in experiment (3) (*a*) and (*b*) and its corresponding controls. Thus in the control observations in experiment (5), when 0·1 c.c. of each of the three antisera concerned was tested separately with 0·00005 gramme homologous protein, it was found that the largest deposit was given by (*a*) antiserum No. 47, and the smallest by (*c*) antiserum No. 48.

Conclusions.

These observations reinforce our previous conclusions* regarding certain phenomena of precipitin reactions by showing :—

(1) That the homologous protein is capable of throwing out of solution many times its weight of precipitum, and

(2) That, in the interaction of minute quantities of homologous protein with large amounts of antiserum, the protein is not completely removed from the superfluid, but that sufficient remains to produce a second, and also to produce a third interaction with similar large amounts of fresh antiserum.

It is apparent, therefore, that the serum (antiserum) of a rabbit, immunised with egg-white or with blood serum, acquires the capacity, not so much of precipitating, as of being precipitated by the homologous protein ; in other words, that the antiserum, and not the homologous protein, is the main source of "precipitable" substance.

We desire to acknowledge the courtesy of Professor Anderson Stuart in placing his laboratory at our disposal.

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* Welsh and Chapman, 'Roy. Soc. Proc.,' B, vol. 78, p. 312, 1906.

Observations upon Phagocytosis carried out by Means of Melanin to Ascertain more particularly whether the Opsonic Index is Identical with the Hæmophagocytic Index.

By S. G. Shattock and Leonard S. Dudgeon.

(Communicated by Professor J. Rose Bradford, For.Sec.R.S. Received December 21, 1907,—Read February 6, 1908.)

(From the Pathological Laboratories, St. Thomas's Hospital.)

The following work was taken up in the belief that interesting information might be obtained by the substitution of a finely divided inert substance in place of bacteria, wherewith to study the phagocytic index in particular infective diseases. The substance which we selected was melanin obtained from the eye of the ox.

The questions which we set ourselves to test by this method we may put *seriatim* ; and we may, after each, recount the particular observations bearing upon it, and give the conclusions to be drawn from them.

Question 1.—Will an increased phagocytosis of melanin take place if melanin is added to the blood of a patient suffering from an infective disease, as compared with normal blood; or if melanin is presented to normal washed leucocytes in an immune serum, as compared with a normal serum ?

To give first the results of the simpler of these two observations. The method consisted in drawing up 1 volume of blood into the capillary end of a pipette, and, immediately after this, 1 volume of citrated salt solution (1 per cent. sodium citrate, 9 per cent. salt), and thirdly 1 volume of melanin, suspended in salt solution.

The three volumes were discharged from the tube by means of a rubber teat fixed to the wider end, mixed in a watch glass in the usual way, and finally drawn up the capillary portion of the pipette, the fine end of which was then sealed in the flame, and the pipette laid horizontally in the incubator at 37° C. for 20 minutes; the blood film was thereupon prepared as already described. In our later observations we simplified this technique by using only two volumes: viz., 1 volume of blood and 1 volume of melanin suspended in *citrated* salt solution, instead of in simple salt.

Summary and Conclusions drawn from the Observations made to Test the First Part of Question (1).

In a total of 32 cases, the phagocytic index is below par in 8; at par in 7; and above par in 17, ascending to 2, 3, 4, 5, 6, and even 8.

It might be at first assumed that the heightened index showed that the increased phagocytosis is due to the presence of a cytotropic substance exclusively, seeing that melanin, not being bacterial, cannot (so it might be supposed) be susceptible to the action of a bacteriotropic or opsonic substance. This, however, is not the case. The proof of this we will adduce later on, but briefly it consists in this, viz., that an immune serum can be largely *de*opsonised by means of melanin if the latter is used to saturation. And if melanin can deopsonise, it can only do so by becoming opsonised. We believe that in both cases the result so obtained is mechanical; the finely divided substance, though inert, entangling or becoming invested with the still more finely particulate material, of which the opsonin would thus appear to consist.

In the six following observations, the ordinary method of estimating the opsonin index was carried out, *i.e.*, normal washed leucocytes were used: (1) in normal serum; (2) in the patient's serum.

The cases comprised:—Acute pneumonia, lymphangitis, pneumonia, acute pneumonia with pleural effusion, urinary fever (colon bacillus infection), empyema.

Remarks on the Six Foregoing Observations.

The index in the third and fourth cases was taken by Wright's method, and also by the simpler one of using normal blood against the blood of the patient. The index obtained by the second method is in both cases higher, being 1·2, as compared with 1; and 4·7 as compared with 1.

Question 2.—Will *saturation of an immune serum* with melanin remove the "opsonin" and reduce the phagocytosis of normal cells against either the bacillus or a suspension of melanin, or against both? That is to say, how far are "opsonisation" and "deopsonisation" strictly specific?

In the following observation the action of the patient's cells in the presence of melanin is compared in the patient's serum, and in the patient's serum after saturation with melanin. The serum was digested with a thick suspension of melanin for 2½ hours, at 37° C.

CASE.—Acute appendicitis; peritonitis; due to *B. coli.*

1 vol. of the patient's washed cells	50 cells
1 ,, ,, serum	contained
1 ,, melanin	68 granules.
1 vol. of the patient's washed cells	50 cells
1 ,, ,, serum after saturation with melanin	contained
1 ,, melanin	28 granules.

The phagocytosis was reduced to nearly one-third.

Case.—Pulmonary tuberculosis ; untreated with tuberculin.

A.	1 vol. of normal washed cells	50 cells contained 155 bacilli.
	1 „ patient's serum	
	1 „ tubercle bacilli (in 1·5 salt solution)	

Serum of patient saturated with thick suspension of tubercle bacilli ; digested for 4¼ hours, at 37° C., and centrifuged.

(i)	1 vol. of the clear fluid thus obtained	50 cells contained 46 bacilli.
	1 „ normal washed cells	
	1 „ tubercle bacilli	
(ii)	1 vol. of the clear fluid thus obtained	50 cells contained 2 granules.
	1 „ normal washed cells	
	1 „ melanin	
B.	1 vol. of normal washed cells	50 cells contained 22 granules.
	1 „ patient's serum	
	1 „ melanin	

Serum of patient saturated with thick suspension of melanin ; digested for 4¼ hours, at 37° C., and centrifuged.

(i)	1 vol. of the clear fluid thus obtained	50 cells contained 5 granules.
	1 „ normal washed cells	
	1 „ melanin	
(ii)	1 vol. of the clear fluid thus obtained	50 cells contained 113 bacilli.
	1 „ normal washed cells	
	1 „ tubercle bacilli	

Case.—Pulmonary tuberculosis ; untreated with tuberculin.

A.	1 vol. of normal washed cells	50 cells contained 170 bacilli.
	1 „ patient's serum	
	1 „ tubercle bacilli	

Serum of patient saturated with an equal portion of a very thick suspension of tubercle bacilli in 1·5-per-cent. salt solution ; digested for 2¼ hours, at 37° C., and centrifugalised.

(i)	1 vol. of the clear fluid so obtained	50 cells contained 13 bacilli.
	1 „ normal washed cells	
	1 „ tubercle bacilli	
(ii)	1 vol. of the clear fluid so obtained	50 cells contained 5 granules.
	1 „ normal washed cells	
	1 „ melanin	
B.	1 vol. of normal washed cells	50 cells contained 18 granules.
	1 „ patient's serum	
	1 „ melanin	

Serum of patient saturated with a very thick suspension of melanin; digested for $2\frac{1}{4}$ hours, at 37° C., and centrifugalised.

(i) 1 vol. of the clear fluid so obtained	50 cells contained 6 granules.
1 „ normal washed cells	
1 „ melanin	

(ii) 1 vol. of the clear fluid so obtained	50 cells contained 56 bacilli.
1 „ normal washed cells	
1 „ tubercle bacilli	

Summary and Conclusions from the foregoing Group of Observations.

In Observation 1.—The saturation with melanin of the immune serum from a case of acute peritonitis reduced the phagocytosis (using normal cells) from 68 granules of melanin to 28 granules. This result shows that a large amount of opsonin has been removed by the melanin, the phagocytosis being reduced to 1/3.

In Observation 2.—Pulmonary tuberculosis, untreated with tuberculin, when the serum was saturated with melanin the phagocytosis (using normal cells) was reduced from 22 granules of melanin to 5 granules, showing that a large amount of deopsonisation towards melanin had been brought about; the opsonin was reduced to 1/4.

Towards tubercle bacilli the phagocytosis (using normal cells) in the patient's serum was 155 bacilli in 50 cells. After saturation with tubercle bacilli the phagocytosis was reduced to 46 bacilli.

How far did the saturation with tubercle bacilli reduce the phagocytosis of melanin? The phagocytosis of melanin was reduced from 22 granules to 2 after saturation of the serum with *tubercle bacilli, i.e.,* the opsonin was reduced to 1/11.

How far did the saturation with melanin reduce the phagocytosis towards tubercle bacilli? The reduction here is from 155 to 113. This is considerably less than will appear from the following observation:—

In Observation 3.—The serum used was from a case of pulmonary tuberculosis, untreated with tuberculin. When the serum was saturated with melanin the phagocytosis (using normal cells) was reduced from 18 granules of melanin to 6 granules, showing that a large amount of deopsonisation towards melanin had been brought about.

As in the preceding observation the opsonin was reduced to 1/3. Towards tubercle bacilli the phagocytosis (using normal cells) in the patient's serum was 170 bacilli in 50 cells. After saturation with tubercle bacilli the phagocytosis was reduced to 13 bacilli.

How far did the saturation with tubercle bacilli reduce the phagocytosis of melanin? The phagocytosis of melanin was reduced from 18 granules to 5 granules after saturation of the serum with *tubercle bacilli.* As it was reduced after saturation with *melanin* from 18 to 6, the reduction is the same; *i.e.*, the tuberculous serum is deopsonised towards melanin, equally by saturation with tubercle bacilli and with melanin.

How far did the saturation with melanin reduce the phagocytosis towards tubercle bacilli? The reduction here is from 170 to 56. It will be noticed that the proportion here is very nearly the same as the reduction in phagocytosis towards melanin after saturation with melanin; the phagocytosis is reduced to 1/3.

Although the fall is only from 170 to 56 as compared with that from 170 to 13 (after saturation with tubercle bacilli), it is clear, nevertheless, that a large amount of the "tubercular opsonin" has been removed by the melanin; and to this extent the opsonin is not specific to the tubercle bacillus.* As already observed the fact than an immune serum can be *de*opsonised by means of melanin shows that melanin can be opsonised.

The opsonisation and deopsonisation are probably in this case mechanical, the finely divided melanin, though inert, entangling or becoming invested with the still more finely particulate material of which the opsonin would thus appear to consist.

Question 3.—Will *heating an immune serum* reduce the phagocytosis towards a suspension of melanin in the same way that it is known to do towards a suspension of a bacterium causing a disease?

The serum in the four following observations was heated at 60° C. for 10 minutes:—

Cases: Erysipelas, acute pleurisy, streptococcus pyæmia, erysipelas.

Summary and Conclusions from the Observations made to Test the Preceding Question.

The observations show clearly that the phagocytosis of *normal* cells is greatly reduced towards melanin, in immune serum that has been heated, as it is towards bacteria.

This is a corollary of the observations recorded under Question 1, which show that in immune serum the phagocytosis of melanin may be largely increased, *i.e.*, that melanin is capable of being opsonised. So, when opsonin is removed by heating the immune serum, the phagocytosis of normal cells is notably reduced.

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* Muir and Martin ('Roy. Soc. Proc.,' May, 1907) conclude that there is present in an immune serum a specific or immune (thermostable) opsonin, and in addition a normal (thermolabile) opsonin. The markedly increased phagocytosis of melanin in immune blood would, on such a view, imply that this was due solely to the increase of a "normal opsonin." In the *de*opsonisation of an immune serum by melanin, the opsonin removed would be, on the same supposition, "normal," however much increased in amount. The authors cited show, indeed, that the thermolabile, "common," or non-specific opsonin may be removed from the same immune serum by various different micro-organisms.

The indices, commencing with that showing the least reduction, run :—

0·67, 0·23, 0·16, 0·16.

Question 4.—Will an increased phagocytosis of melanin take place if melanin is presented to the washed leucocytes of an immune blood in *normal serum*, as compared with normal washed leucocytes in *normal serum* ?

The cases selected comprised the following : acute erysipelas, chronic bone abscess, acute pneumonia, lymphangitis, streptococcal pyæmia, acute cellulitis, acute peritonitis. Of some of these more than one example was used.

Summary and Conclusions from the Observations made to test the Preceding Question.

In the case where melanin is presented to normal washed cells and to the patient's washed cells, both in *normal serum*, the phagocytosis is, in some, below par ; in others, above ; in others, at par.

The indices run :—

0·46, 0·7, 1, 1, 1, 1·1, 1·4, 1·5, 1·6, 1·8, 2·9.

Question 5.—Will the phagocytosis be the same if melanin is presented to the washed cells of the patient, and to washed normal cells, in *immune serum* ?

In the following experiments, the action of normal cells and patient's cells was tested in the patient's serum.

Case.—*Streptococcus pyogenes* pyæmia.

Index......... 1·7.

Case.—Acute pneumonia ; 5th day of disease ; male, æt. 23 ; temperature 103° F.

Index......... 3·3.

In each of these observations, the work done by the patient's cells is greater than that done by normal cells, the indices being 1·7, 3·3.

In the four following observations, the phagocytosis of leucocytes from different cases of *Bacillus coli* infection, in the same immune serum is set forth, as compared with that of normal cells in the same immune serum. The immune serum was from a case of bone abscess in which the colon bacillus was present. The patient had been treated with anti-serum and also with vaccine.

Case.—*B. coli* infection of the urinary tract ; treated with anti-coli serum and with vaccine.

Index......... 0·9.

CASE.—Bone abscess in which *B. coli* was present; treated with anti-coli serum and with vaccine.

Index......... 1.

CASE.—Acute appendicitis; treated with anti-coli serum and with vaccine.

Index......... 1·2.

CASE.—Acute peritonitis; treated with anti-coli serum.

Index......... 2·4.

The indices in these observations run:—

0·9, 1, 1·2, 2·4.

In the last of them the *patient's cells are doing considerably more work than normal cells in the same immune serum.*

In the three following observations, the cells from a standard case of acute pneumonia were tested against normal cells in immune sera from cases of different disease:—

CASE.—Empyema.

1 vol. of normal washed cells,
1 „ patient's (empyema) serum,
1 „ melanin.

1 vol. of washed standard pneumonia cells
1 „ patient's (empyema) serum,
1 „ melanin.

Index......... 1·3.

CASE.—Urinary fever (*B. coli* infection).

1 vol. of normal washed cells,
1 „ patient's (urinary fever) serum,
1 „ melanin.

1 vol. of washed standard pneumonia cells,
1 „ patient's (urinary fever) serum,
1 „ melanin.

Index......... 1·7.

CASE.—Acute pneumonia.

1 vol. of normal washed cells,
1 „ patient's (pneumonia) serum,
1 „ melanin.

1 vol. of washed standard pneumonia cells (from another patient),
1 „ patient's (pneumonia) serum,
1 „ melanin.

Index......... 6·5.

In all these three observations, it will be seen that the washed pneumonia cells are doing more work than washed normal cells in the immune serum of three different patients, the most marked difference being that where pneumonia serum was selected.

The indices run :—

1·3, 1·7, 6·5.

In the following observations, the action of normal cells and patient's cells, from a case of acute general peritonitis, was tested in the serum of another patient suffering from chronic pulmonary tuberculosis.

1 vol. of normal washed cells,
1 „ tuberculous serum,
1 „ melanin.

1 vol. of patient's washed cells (acute peritonitis),
1 „ tuberculous serum,
1 „ melanin.

Index......... 1·4.

This observation, like the three that precede it, shows that the washed cells, from a case of acute infection, are more active that normal cells, in the serum of a patient suffering from another disease, the index being 1·4.

In the two following observations, the action of normal washed cells is compared with the action of the patient's washed cells, in the presence of melanin, in the patient's serum, heated at 58° C. for 15 minutes.

Index......... 0·9.

Case.—Acute cellulitis of leg.

Index.......... 2.

In the two following observations, the action of normal washed cells is compared with the action of standard-pneumonia cells, in the heated serum of two other patients.

Case.—Acute pneumonia.

1 vol. of normal washed cells,
1 „ heated pneumonia serum,
1 „ melanin.

1 vol. of standard pneumonia washed cells (*i.e.*, from another case of pneumonia).
1 „ heated pneumonia serum,
1 „ melanin.

Index......... 1.

Case.—Empyema.

1 vol. of normal washed cells,
1 „ heated empyema serum,
1 „ melanin.

1 vol. of standard pneumonia washed cells,
1 „ heated empyema serum,
1 „ melanin.
(Serum heated at 60° C. for 20 minutes.)

Index......... 1·6.

In the following observation, the action of normal washed cells is compared with the action of the same standard pneumonia cells as used in the preceding observations, in *normal heated serum.*

1 vol. of normal washed cells,
1 „ normal heated serum,
1 „ melanin.

1 vol. of standard pneumonia washed cells,
1 „ normal heated serum,
1 „ melanin.

Index......... 2.

In the five preceding observations, in the first four of which washed normal cells were tested against washed immune cells in heated immune serum, and in the fifth in normal heated serum, the indices run :—

0·9, 1, 1·6, 2, 2.

In two the immune cells are doing more work than normal cells in the heated immune serum, and the same is true in the case of the heated normal serum.

In the following observations, *colon bacilli* were substituted for melanin, and the cells from a case of streptococcal puerperal fever, with multiple intramuscular and subcutaneous abscesses, were used in coli serum against normal cells in the same coli serum.

The patient from whom the cells were taken died within 48 hours after the blood was taken. The blood was observed to be very watery when taken, and showed agglutination of the red cells.

CASE.—Urinary fever ; *B. coli* infection.

1 vol. of normal washed cells,
1 „ „ serum,
1 „ colon bacilli.

1 vol. of normal washed cells,
1 „ patient's serum (*B. coli* infection),
1 „ colon bacilli.

Index......... 2·4.

This index shows that the patient's serum is markedly active, *i.e.*, it contains a good amount of an opsonin which will prepare the colon bacillus for ingestion.

In the next place, the cells from a patient suffering from puerperal fever (streptococcal) were substituted for normal washed cells, thus:—

1 vol. of normal washed cells,
1 ,, patient's serum (colon infection),
1 ,, colon bacilli.

1 vol. of washed cells of patient suffering from puerperal fever,
1 ,, patient's serum (colon infection),
1 ,, colon bacilli.
Index......... 0·5.

This observation indicates that, although in colon serum, colon bacilli, when presented to normal washed cells, may give a good index (2·3), when presented in the same serum to the washed cells of another patient suffering from puerperal fever, the index fell to 0·5.

This can only mean that the cells from the puerperal patient are incapable of carrying out the same amount of ingestion as normal cells in the same colon serum against the same colon bacilli, *i.e.*, the cells cannot make use of the phagocytic opportunity offered to them. Their decreased activity may be ascribed to damage sustained whilst in the circulating blood, or to the exhaustion brought about by the forced production of antibodies under the same circumstances.

It may be inferred from these data that a low index (as reached by the usual method) might be found still lower were the *patient's cells* used in the patient's serum, in place of using *normal cells* in the patient's serum against normal cells in normal serum.

1 vol. of normal washed cells,
1 ,, ,, serum,
1 ,, colon bacilli.

1 vol. of patient's washed cells (puerperal fever),
1 ,, normal serum,
1 ,, colon bacilli.
Index......... 0·56.

This extension of the foregoing observation shows that the cells from the same case of puerperal fever do only half the work in normal serum against colon bacilli which normal cells do in the same normal serum against colon bacilli. This is the same proportion which was obtained by presenting:

(1) Normal cells, and
(2) The same patient's cells,

in the patient's serum, where the patient's cells are doing only half the work of normal cells.

In the following experiment, colon bacilli were substituted for melanin;

and the washed cells, from the same case of puerperal fever as the preceding, were tested against normal cells in the patient's serum.

Case.—Puerperal fever; *Streptococcus pyogenes;* death with pyæmia.

1 vol. of normal washed cells,
1 „ „ serum,
1 „ colon bacilli.

1 vol. of normal washed cells,
1 „ patient's serum (puerperal fever),
1 „ colon bacilli (not from patient).

Index......... 0·8.

1 vol. of normal washed cells,
1 „ patient's serum (puerperal fever),
1 „ colon bacilli (not from patient).

1 vol. of patient's washed cells (puerperal fever),
1 „ „ serum,
1 „ colon bacilli (not from patient).

Index......... 0·3.

This observation shows that the patient's cells (streptococcal infection) are less active in the patient's serum, against colon bacilli, than are normal cells in the patient's serum against colon bacilli. The result corresponds closely with those which precede it, in which a colon serum was used in place of the above patient's serum, but with the same puerperal patient's cells.

Summary and Conclusions from the foregoing Groups of Observations placed under Question 5.

In the observations where melanin was presented to normal washed cells and to a patient's washed cells, in the patient's own serum, or in the serum of another patient suffering from another infective disease, the indices run:—

0·9, 1, 1·2, 1·3, 1·4, 1·7, 1·7, 2·4, 3·3, 6·5.

In *heated immune serum*, the washed immune cells against normal washed cells give indices of:—

0·9, 1, 1·6, 2.

In *heated normal serum*, the index, in the single observation made, was 2.

In the majority of the cases, the patient's cells take up more melanin, or more bacilli, than do normal cells in immune serum, whether the immune serum be that of the patient or that of another patient suffering from another infective disease; the higher indices run:—

2·4, 3·3, 6·5.

When the patient's cells are compared with normal cells in *normal serum*, the indices range from

0·46 to 2·9.

The action of the patient's cells would thus appear to be a factor which requires consideration if a full estimate of the patient's hæmophagocytic resistance is to be arrived at.

Were the patient's cells indifferent, normal cells would ingest to the same extent as the patient's cells, in the patient's serum. This is not the case. The patient's cells may be less active, or they may be more active than the normal.

The conclusion to which the observations lead is that the calculation of the opsonic value of the patient's serum alone does not give a full estimate of the patient's hæmophagocytic resistance. It may be too high or too low, or, as a coincidence, it may exactly represent it.

In what may this greater activity of the patient's cells consist? The greater activity might be ascribed to an elaboration and excretion of opsonin by the patient's leucocytes in the patient's serum, which brings about a still further increased preparation of melanin in the already opsonised serum.

This would mean that the patient's leucocytes, instead of playing only the secondary part of ingesting prepared bacteria, both prepare and then ingest them.

To gauge the curative capacity of the patient's serum only, the method in commoner use is doubtless the correct one, for normal cells are used in the patient's serum against normal cells in normal serum. But the question may be raised whether the action of the patient's cells *should* be considered. For if their greater activity is due only to a further secretion of opsonin, in observations carried out *in vitro*, it may be urged that this exalted phagocytic index does not represent that of the circulating blood, but is too high. The patient's cells are transferred to the patient's serum in relatively abnormal numbers, and, during the 20 minutes' incubation, they may be elaborating from the patient's serum a further amount of opsonin which overcharges the serum in the capillary tube and brings about a fictitious degree of phagocytosis.

If any substance continues to be produced in the serum, *in vitro*, it must be an opsonin. It cannot be a substance that would stimulate the cells to greater activity: we cannot suppose that the cells produce and shed into the serum a "stimulin" in order to stimulate themselves! The patient's cells produce a further amount of opsonin, or they are simply "more active" than are normal cells.

One must remember that the process of thrice washing the cells in salt solution does not wash out the leucocyte, it only washes the surface of it.

One difficulty that arises in testing the alternatives just stated will be obvious:—During the process of incubation, *in vitro*, two phenomena may be proceeding simultaneously: the cells may be simply ingesting, or they may be producing opsonin and ingesting. If the phagocytosis increases upon allowing further time, it has to be determined whether this is due to further opsonification, or simply to the fact that the cells are allowed a longer time in which to work. And even the termination of further phagocytosis, after an extreme time-allowance, might mean equally either a termination of further opsonin production, or that the cells had taken up all for which they had capacity.

Our observations bring out that the immune or active cells, as compared with normal cells, although they usually do more work in both immune and in normal serum, do more in the immune than in the normal. This points to some interaction between the patient's cells and the patient's serum; it indicates that there is something in the patient's serum which is in excess, or which is not present, in normal serum. If we agree to call this "something" a stimulin, its presence will not, *per se*, account for the difference. For such a substance would equally stimulate the normal cells to do the same amount of work as the immune cells in the immune serum—which is not the case.

In heated immune serum, again, the immune cell does more work than the normal: so that, whether the substance left in the serum after heating is specifically bacteriotropic and unable to affect the melanin or cytotropic (stimulin), it is clear that the immune cells behave differently from the normal. We are led to hold, therefore, that the immune cell, as a cell, is in many cases more active, more irritable, or more sensitive, than the normal, as it may be less active than the normal cell, or active to the same degree.

The immune cell has acquired a heightened activity in the body in response to the increased function demanded of it to cope with the infective process; its full reserve power has been called out. The fact that more phagocytosis occurs in immune serum with immune cells than with normal cells indicates that, in the living body, from a similar interaction, the patient's cells are likewise doing more work than would normal cells, if we imagined, *e.g.*, the whole of the patient's leucocytes suddenly replaced in his own plasma by normal cells.

Question 6.—Is opsonin produced, *in vitro*, by the patient's cells in the patient's serum?

The only thing that would vitiate the foregoing conclusion would be evidence of an additional formation of opsonin, *in vitro*, during the 20 minutes in which the capillary tube containing the immune serum, the immune cells, and the melanin, or bacilli, was being incubated.

It is conceivable that the immune serum contains a precursory substance—an opsinogen—which is converted into opsonin by a ferment produced by the cells, and that the increased phagocytosis of immune cells in immune serum is due to their greater secreting activity, *i.e.*, to their increased production of the converting substance; and that when melanin or bacteria are presented *in vitro*, this interaction is set going, or, rather, restarted in the drawn blood.

Observation.

Case.—Empyema.

1 vol. of blood,
1 „ citrated salt suspension of melanin.

Four tubes were prepared, and incubated for different periods, with the following results:—

15 minutes	9 granules of melanin in 50 cells.		
1 hour	21	„	„
2 hours	36	„	„
4 „	76	„	„

In such an observation it is impossible to determine whether the progression is due to further formation of opsonin or to the increased time allowed for the continuance of phagocytosis.

When bacteria are used in place of melanin, this progression may be more highly pronounced, as is shown in the three following observations:—

Case.—Acute cellulitis; temperature, 102° F.

1 vol. of patient's blood,
1 „ citrated salt suspension of colon bacilli.

Incubated 20 minutes .... 50 cells contained 126 bacilli.

1 vol. of patient's blood,
1 „ citrated salt suspension of colon bacilli.

Incubated 30 minutes .... 50 cells contained 195 bacilli.

Case.—Urinary infection due to colon bacillus; untreated with anti-serum or vaccine.

1 vol. of patient's blood,
1 „ citrated salt suspension of colon bacilli.

Incubated 20 minutes .... 50 cells contained 115 bacilli.

1 vol. of patient's blood,
1 „ citrated salt suspension of colon bacilli.

Incubated 30 minutes .... 50 cells contained 165 bacilli.

Case.—Urinary infection due to colon bacillus; treated with anti-serum and vaccine.

1 vol. of patient's blood,
1 „ citrated salt suspension of colon bacilli.

Incubated 20 minutes .... 50 cells contained 116 bacilli.

1 vol. of patient's serum,
1 „ citrated salt suspension of colon bacilli.

Incubated 30 minutes .... 50 cells contained 196 bacilli.

It is worth while to notice that such a progression does not *necessarily* occur under the same conditions of experiment.

Observation.

Case.—Suppuration of thumb.

1 vol. of blood,
1 „ citrated salt suspension of melanin.

Four tubes were prepared, and incubated for different periods, with the following results:—

15 minutes	31 granules of melanin in 50 cells.
30 „	28 „ „
1 hour	29 „ „
2 hours	31 „ „

Here it may be inferred that the immune cells were of lowered activity. This we know to be so in some cases, where they do less work in their own immune serum than do normal cells.

Observation.

Normal Blood.—In the case of normal blood, a slight progression in phagocytosis may be observed:—

1 vol. of normal blood,
1 „ citrated salt suspension of melanin.

Four tubes were prepared, and incubated for different periods, with the following results:—

15 minutes	18 granules of melanin in 50 cells.
30 „	16 „ „
1 hour	26 „ „
2 hours	31 „ „

Here, again, the progression might be due to a further formation of opsonin, or to the further time allowed for the process of ingestion.

The progressive phagocytosis in normal blood is more forcibly shown in the following observation, where colon bacilli are used in place of melanin:—

1 vol. of normal blood,
1 „ citrated salt suspension of colon bacilli.

Incubated 20 minutes .... 50 cells contained 151 bacilli.

1 vol. of normal blood,
1 „ citrated salt suspension of colon bacilli.

Incubated 30 minutes .... 50 cells contained 399 bacilli.

In considering whether the index obtained by using immune cells in immune serum, *in vitro*, correctly represents the phagocytic resistance of the patient's blood, *i.e.*, of his serum and his cells, as distinguished from the resistance brought about by his serum alone, the question with which we are essentially concerned is whether a further formation of opsonin occurs *in vitro*, or whether the work done by the cells is done by means of the opsonin which was present in the blood when the latter was drawn.

We endeavoured to determine this by means of the following experiments:—

One volume of washed immune cells was thoroughly mixed with one volume of immune serum, using larger volumes than usual. The mixture was then drawn up into three capillary tubes of the usual calibre, and incubated for 20 minutes, 30 minutes, and 1 hour.

At the end of these intervals, an equal volume of citrated salt suspension of living colon bacilli was added to the contents of each capillary tube, mixed, and each tube then incubated for 20 minutes.

By this means, a full supply of active immune cells was allowed to act in immune serum for different periods (without the presence of bacilli); the phagocytosis was then tested in each case after the same time-allowance, viz., 20 minutes.

Case.—Colon infection of urinary tract; treated with anti-serum and vaccine.

1 vol. of immune washed cells and immune serum, incubated 20 minutes,
1 „ citrated salt suspension of colon bacilli.
50 cells contained 73 bacilli after 20 minutes' incubation.

1 vol. of immune washed cells and immune serum, incubated 30 minutes,
1 „ citrated suspension of colon bacilli.
50 cells contained 74 bacilli after 20 minutes' incubation.

1 vol. of immune washed cells and immune serum, incubated 1 hour,
1 „ citrated suspension of colon bacilli.
50 cells contained 81 bacilli, after 20 minutes' incubation.

Summary and Conclusions from the foregoing Group of Observations.

It will be evident from the concluding observation that no difference in phagocytosis is brought about within 20 minutes, in different samples of the same immune serum, mixed with the immune cells from the same patient, when bacilli are presented, after periods of incubation of 20 minutes, 30 minutes, and 1 hour; the numbers of bacilli ingested by 50 cells, being:—

73, 74, 81.

This crucial experiment proves that the marked difference in the phagocytosis observed when bacilli are added to immune blood, and incubated for different periods, must be attributed, not to further opsonification of the serum, but to the increased time allowed for the cells in which to ingest. The practical conclusion we draw is, that in order to estimate the full phagocytic resistance of the patient's blood, the cells should be taken into account as well as the serum.

The method of making such an estimate is considerably simpler and much more rapid than the estimation of the opsonic value of the serum only, which entails washing normal cells and collecting blood in order to obtain both normal serum and the serum of the patient.

It is merely necessary to draw up 1 volume of the patient's blood and 1 volume of citrated salt suspension of the micro-organism to be used, into the capillary tube, to mix, incubate for 20 minutes, spread the film, and compare the phagocytosis with that of a volume of normal blood similarly treated,—a slight modification of the technique practised by Leishman.

The washed corpuscles, moreover, sometimes tend to cohere in clumps. The central cells of such clusters would fail to reach bacilli presented in the fluid. This is probably one explanation of the discrepancies obtained in the numeration of ingested bacilli, even in neighbouring fields of the same slide.

It has been pointed out elsewhere in this communication that although the action of the immune phagocyte is usually higher than that of the normal cell, yet it may be lower, or it may be equal to it. *The cells vary in value, like the serum, and the only method of arriving at a correct estimate of the patient's hæmophagocytic resistance is to allow the immune cells to work in the immune serum.*

By the method more commonly employed in this country, too low an index is obtained if the patient's cells are acting above the normal level, and too high an index if they are acting below it.

A Contribution to the Study of the Mechanism of Respiration, with Especial Reference to the Action of the Vertebral Column and Diaphragm.

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(Communicated by Professor Sir T. Clifford Allbutt, K.C.B., F.R.S.
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In studying the alterations which occur in the shape, size, and position of the internal organs as the result of their functional activity, previous observers have worked at a disadvantage. During the past nine years X-rays methods, though indicating an advance in our knowledge of abdominal and thoracic visceral movements, have not been of absolute utility, since the rays, being divergent, produce magnification of the shadow of the object. Hence, exact measurements have been unattainable.

In the present investigation the chief results have been obtained by means of Groedel's orthodiagraph, which Dr. Hugh Walsham and myself have been the first, to our knowledge, to work with in this country, and of which we have already published a detailed description.* By means of this instrument it is possible, with almost mathematical accuracy, to measure motionless objects which lie in a plane parallel with the vertical transverse plane of the body, and to measure moving objects with greater approach to exactitude than can be obtained in any other manner.

SUMMARY OF THE RESULTS OF THE INVESTIGATION.

I. *Results obtained by Orthodiagraphic Measurement of Changes in the Trunk which occur during Respiration.*

One hundred healthy subjects, of ages varying between 15 and 35, were examined, the average measurements being recorded as follows :—

(1) The neck is shortened 10 mm., and widened, on the right side 9 mm., on the left side 7 mm.

(2) The shoulders are raised on the right side to a greater extent than on the left, the average on the right being 16 mm., that on the left 14 mm.

(3) The presternum moves 30 mm. in an upward, and 14 mm. in a forward, diameter.

(4) The clavicles execute a combined upward, forward, and outward

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* 'Brit. Med. Journ.,' September 14, 1907, p. 651.

movement, the vertical range of their inner ends on the right side being 28 mm., on the left side 27 mm.; of their outer ends, on the right side 21 mm., on the left side 16 mm. The divergence from the median line is, on the right side 7 mm., on the left side 6 mm.

(5) The meso-metasternal articulation either may remain in the same horizontal plane, or may rise as much as 46 mm. The average ascent is 28 mm.

(6) Widening of the infra-costal angle occurs, the interval between the costal margins, measured on each side at a level of 30 mm. below the meso-metasternal articulation, being increased by 26 mm.

(7) The trunk is widened at the level of the meso-metasternal plane 9 mm. on the right side, and 8 mm. on the left, and midway between the meso-metasternal plane and lowest point of the costal margin 9 mm. on the right side, and 11 mm. on the left.

(8) The umbilicus is retracted and drawn upwards in deep respiration for a distance of 13 mm., on account of the active recession of the abdominal wall produced by the contraction of the abdominal muscles, which, in this phase of respiration, act as antagonists of the spinal muscles. The upward displacement of the umbilicus is usually to the right, but may be median or to the left, the lateral deviation being 7 mm.

(9) The heart and pericardium together undergo important changes in size and position as a result of the respiratory movements, being lengthened and narrowed in inspiration, shortened and widened during expiration.

(10) The pericardium, at the level of its attachment to the central tendon in the adult, measures 80 mm. in antero-posterior diameter.

II. *The Movement of the Vertebral Column in Respiration.*

I can find no reference to this movement in eight of the latest and best known text-books of physiology. That this movement is actual and of mechanical advantage in breathing can be verified by visual and orthodiagraphic examination. In the latter the subject is rotated through an angle of 30° to 45° into the "lateral oblique" position. The shadow of the vertebral column is seen clearly separated from that of the pericardium and great vessels by a transradiant triangle, the base of which is formed by the upper surface of the diaphragm. On inspiration the posterior wall of this triangle, formed by the vertebral column, recedes, to a greater extent below than above, and so opens out the interval from before backwards. With subsequent expiration the spine advances.

Explanation of the Spinal Respiratory Movement.—A general rectification of the curve of the thoracic spine takes place. In eight orthodiagraphic examinations of healthy adults, in deep inspiration, the average antero-posterior range of movement was as follows:—

At upper aperture of thorax opposite 1st thoracic vertebra, 6 mm.

Midway between upper aperture of thorax and level of diaphragm opposite 5th thoracic vertebra, 7·5 mm.

At level of posterior part of diaphragm opposite 10th thoracic vertebra, 9 mm.

These measurements show that on the average the spinal column is most displaced towards the lower part of the thoracic curvature, and that rectification lessens from below upwards. Individual differences, however, are not infrequent. This straightening, which occurs especially in that segment of the spine which articulates with the 6th, 7th, 8th, and 9th ribs, happens as a consequence of the backward push of the sternal ribs when the sternum is raised upwards and forwards by contraction of the cervical and thoracic muscles, thus bringing a larger costal arc into the place previously occupied by a smaller one, and so increasing the antero-posterior diameter of the thorax. Upon the ribs assuming a position of less obliquity, the spinal column, being far more limited in its possible range of movement than the sternum, on account of its multiple attachments can only execute a fraction of the sternal movement. Towards the end of inspiration, as the movement of the sternum reaches its dynamic limit, the remainder of the force of the respiratory cycle is spent upon the spine, which accordingly, during the latter half of inspiration, shows progressive mobility.

In the lateral oblique position the apparent movement of the vertebral column is 6 to 9 mm.; the real movement may be readily calculated by means of a mathematical formula.

Importance of the Spinal Movement.—The antero-posterior enlargement of the thoracic cavity produced by simultaneous extension of the thoracic vertebræ in deep breathing has an important influence upon the aeration of the apices of the lungs, the forward and upward movement of the thorax, together with backward movement of the spine, being of far greater value than the lateral movement in promoting free access of air.

III. *Anatomical Dissimilarity of the Two Halves of the Diaphragm.*

Anatomically the two halves of the diaphragm differ in size and shape, the right half being the larger and the more powerful, the reason being that this half has to overcome the resistance of the mass of the liver, whereas the stomach is much more easily compressed by the left half. Functionally, although the two halves of the diaphragm are of unequal size and strength, yet, owing to the difference in resistance on the two sides, their range of movement is but little dissimilar, the difference, if any, being usually in favour of the right half.

IV. *The Means by which the Diaphragm is supported.*

In another paper I have dealt with the superior, or thoracic, and the inferior, or abdominal, supports of the diaphragm.

V. *Level of the Diaphragm.*

(1) *After Death.*—The cadaveric position of the diaphragm indicates only the position of expiration. From the results of an examination of 80 dissecting-room and post-mortem-room cases, I find that the average highest point of the dome of the diaphragm is situate, on the right side at the level of the upper border of the 5th rib in the mid-clavicular line; on the left side, in the mid-clavicular line at the lower border of the 5th rib. This corresponds with the results of orthodiagraphic measurement in bodies of healthy persons who have met with sudden death.

(2) *During Life.*—Owing to the great variability in position of the landmarks usually adopted in measuring the range of the diaphragm, I have taken the meso-metasternal articulation, *i.e.*, junction between gladiolus and ensiform, as the basis from which to measure, and a line drawn horizontally through this point—the meso-metasternal plane—as the plane to which the level of the rise and fall of the diaphragm may be referred, and all orthodiagraphic measurements of the position of the domes and central tendon have been taken in millimetres above and below this plane.

VI. *Absolute Range of Movement of the Diaphragm.*

The absolute range of movement of the diaphragm between deep inspiration and expiration in the adult male is, on the right side 34 mm., on the left side 32 mm. The range in adult females amounts to 27 mm. on the right side, and 25 mm. on the left side, making the total average range 30 mm. on the right side, and 28 mm. on the left.

The fact that these figures are only about half as great as those previously given* is due to the greater accuracy of orthodiagraphic measurement. In quiet respiration the total average movement is 12·5 mm. on the right side, and 12 mm. on the left. Hence this movement is approximately equal on the two sides, whilst in deep breathing, the excursion is, for most people, slightly greater on the right side than on the left. In diagnosis, the movement in deep respiration is the important one to observe.

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* The 'Lancet,' June 27, 1903, p. 1802.

VII. *The Costo-phrenic Pleural Reflexion.*

According to the surfaces with which it is in contact the parietal layer of the pleura conveniently may be divided into costal, diaphragmatic, and mediastinal portions. The foldings of the pleura constitute three marginal grooves or recesses:—

1. Pericardio-phrenic groove.
2. Pericardio-sternal pleural reflexion.
3. Costo-phrenic pleural reflexion.

1. *The pericardio-phrenic groove* is a shallow recess formed at the junction of the pericardium with the diaphragm, and lodges the plicæ adiposæ.

2. *The pericardio-sternal reflexion* forms the anterior marginal pleural recess.

3. *The costo-phrenic reflexion* must be considered in greater detail, since it facilitates to such a marked extent the action of the diaphragm. It is formed by the meeting of the costal and diaphragmatic portions of the pleura, and, beginning at the lower border of the 6th rib close to the termination of the gladiolus, it passes downwards and outwards, reaching the lateral aspect of the spinal column at the lower border of the twelfth thoracic vertebra. Practically the limit to which the lung descends in ordinary inspiration marks its upper boundary. Here, owing to friction between the pleural surfaces, a line of demarcation, best seen in the recent state, indistinct in the young child, but increasingly definite with advancing age, gradually forms. If evidences of pleurisy are present, not infrequently a ridge of organised lymph, concave on its upper surface, delimits the lower lung margin, forming a groove into which the lung fits. The lower border of the reflexion is wavy or festooned, the festoons being in relation with the intercostal spaces. In a series of 20 male and female subjects, of ages varying from 18 to 56 years, who had died from causes not involving the lungs, the average depth of the costo-phrenic reflexion, measured in the mid-axillary line from lower margin of lung to lowest limit of pleural cavity, on the right side was found to be 8·62 cm., and on the left side 8·34 cm. The greatest interval in the series was 11 cm. on the right side (corresponding to 9 cm. on the left), and the smallest, 5 cm. on the left side (corresponding to 6 cm. on the right). In a female infant aged three months the depth of the reflexion on the right was 1·5 cm., and on the left 1·4 cm.

Function of the Costo-phrenic Pleural Reflexion.—The two serous surfaces constituting the reflexion remain in apposition for a distance varying with inspiration and expiration, and are not separated until the wedge-shaped lower lung margin glides downwards in inspiration and insinuates itself between them. As the lung recedes the surfaces again come together. The diaphragmatic pleura, owing to its elasticity, accommodates itself to contraction of the diaphragmatic circumferential muscle-fibres, and its smooth surface allows it to glide easily over the apposed costal layer, which is fixed in such wise that it cannot be displaced. Owing to negative intra-pleural suction and positive intra-abdominal pressure, together with molecular cohesion of the lubricated pleural surfaces, the pleural union thus formed is of sufficient strength to bind the diaphragm during its action closely to the chest-wall, and, moreover, is of mechanical advantage to the diaphragm, since in function it resembles the band or loop through which a muscle acts in order to change the direction of its line of force.

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The Influence of Temperature on Phagocytosis.

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(Communicated by Dr. C. J. Martin, F.R.S. Received November 18, 1907,—
Read February 27, 1908.)

Recent work has shown that the phagocytosis of micro-organisms is a complex phenomenon, involving at least two factors, viz.: (1) the sensitisation of the micro-organism by the opsonin of the blood serum, and (2) the phagocytic act of the polymorphonuclear leucocyte. The latter function has been known for many years to be markedly influenced by temperature. It appeared desirable, however, to ascertain whether, by the modern quantitative methods employed in opsonic work, some definite relationship could be found to subsist between degree of temperature and degree of phagocytosis. In the first set of experiments no attempt was made to separate the respective functions of sensitisation, and amœboid activity of the leucocyte. Both actions were allowed to proceed simultaneously.

Consequently, in Series I the leucocytes were put in contact with fresh serum and micro-organisms (staphylococci), and incubated together at different temperatures for the same period of time. Thereafter the number of cocci taken up per leucocyte was calculated on stained films in the usual way. The *Staphylococcus aureus* employed was an old laboratory strain.

Series I.—Experiment I.

Temperature.	Cocci per leucocyte with incubation periods.		
	15 mins.	30 mins.	45 mins.
43° C.	15·4	30	30 +
37	13·2	23	25
18	3·1	3·3	7·9

It will be seen that at a temperature of 18° C. the degree of phagocytosis for 30 minutes is not appreciably greater than that for 15 minutes. There is, in fact, a prolonged latent period. This was further brought out in the next experiment, where the temperature remained constant (18° C.) while the incubation periods varied.

Experiment II.

Temperature.	Incubation period.	Cocci per leucocyte.
18° C.	15 mins.	2·9
18	30	3·8
18	45	7·2
18	1 hr.	10·7
18	1 hr. 15 mins.	12·7
18	1 hr. 30 mins.	19·2

For 37° C. this latent period is much shorter. To ascertain this, five-minute intervals were taken as in the following experiment:—

Experiment III.

Temperature.	Incubation period.	Cocci per leucocyte.
37° C.	5 mins.	2·0
37	10	2·7
37	15	4·3
37	20	4·0
37	25	5·6
37	30	4·9
37	35	5·0
37	40	6·5
37	45	8·6
37	50	12·0

The five following experiments showing the influence of temperature on phagocytosis were carried out in a similar way to Experiment I. Coccal emulsions of varying strengths were purposely employed throughout the series:—

Experiment IV.

*I. T. = 15 mins.

Temperature.	Cocci.
41° C.	17·1
37	15·3
17	4·2

Experiment V.

I. T. = 15 mins.

Temperature.	Cocci.
43° C.	15·7
37	12·3
31·5	10·9
25·7	4·0
20	3·2
13	0·8
6·5	?

Experiment VI.

I. T. = 30 mins.

Temperature.	Cocci.
42° C.	8·1
37	9·0
30	8·5
25	6·3
17	3·7

Experiment VII.

I. T. = 33 mins.

Temperature.	Cocci.
43° C.	28·0
37	20·7
31·5	22·3
25·7	18·4
20	10·9
13	9·6
6·5	0·7
0	?

Experiment VIII.

I. T. = 30 mins.

Temperature.	Cocci.
41° C.	21·4
36·6	11·8
30	8·2
17	4·8

From the results quoted above, it will be apparent that the degree of phagocytosis rises, though in a somewhat irregular fashion, as the temperature rises, but the results are not sufficiently constant to enable one to determine with any accuracy such a value for the temperature-coefficient as would allow one to calculate the amount of phagocytosis at a temperature A when the amount at a temperature B is known. The strength of the

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* I. T. = Incubation Time.

coccal emulsion has probably a great bearing on the rate of fall, especially during the upper temperature ranges (*vide* Experiment VI). A fairly constant ratio, however (4—5 : 1), is found to exist between the degree of phagocytosis at 43° C. and that at 18° C.

We have now to determine whether this increased phagocytosis following rise of temperature is due simply to increased activity on the part of the leucocyte. In short, what effect has temperature on the rate of combination of the serum with the coccus?

To settle this point, a second series of experiments was performed. The cocci were sensitised by contact with serum for a fixed time at a certain temperature. Thereafter leucocytes were added and incubation performed at different temperatures. By this method more or less completely sensitised cocci were exposed to the action of the leucocytes.

Series II.

Experiment I.—A moderately thick emulsion of cocci in normal saline was added in equal volume to fresh serum, and kept in contact therewith at a temperature of 37° C. for 20 minutes in a small test-tube. One volume of leucocytes was then incorporated with one volume of the shaken up and partially centrifugalised serum-coccal mixture and incubated at different temperatures for 20 minutes. The result was as follows:—

Temperature.	Cocci per leucocyte.
37° C.	4·4
30	4·4
17	4·3

(The low counts obtained here show that the centrifugalisation of the serum-coccal mixture had been carried a little too far.)

Experiment II.—Technique similar to above.

I. T. = 30 mins.

Temperature.	Cocci per leucocyte.
41° C.	13·8
37	11·8
30	12·2
17	13·5

(Again the fall of temperature had no effect on the amount of phagocytosis.)

Experiment III.—Combination at 37° C. for 20 mins.

I. T. = 15 mins.

Temperature.	Cocci.
37° C.	12·5
17	10·3

Experiment IV.—Combination at 37° C. for 20 mins.

I. T. = 15 mins.

Temperature.	Cocci per leucocyte.
37° C.	5·7
28	5·2
18	5·9
7	3·6

Experiment V.—In this experiment the sensitisation was performed at 18° C. for 20 minutes, and the same coccal emulsion was employed as in Experiment IV, so that the figures obtained are quite comparable.

Temperature.	Cocci per leucocyte.
37° C.	6·2
28	4·2
18	2·6
7	0·8

Experiment VI.—A portion of the coccal emulsion was combined with serum at 37° C. for 20 minutes, and an equal portion combined with serum at 0° C. for the same time. Leucocytes were then added, and the results of phagocytosis at different temperatures compared.

Combination at 37° C.			Combination at 0° C.		
Temperature.	Incubation time.	Cocci.	Temperature.	Incubation time.	Cocci.
37° C.	15 mins.	12·1	37° C.	15 mins.	8·8
20	15	8·6	20	15	0·4

The very great difference between the amounts of phagocytosis at 37° C. and 20° C. after combination at 0° C. offers a marked contrast to what obtains under the same conditions after combination at 37° C. The employment of very thin coccal emulsions, however, and the prolongation of the period of combination with the serum, have the effect of equalising the amounts of phagocytosis at high and low temperatures even after low-temperature combination.

Throughout the experiments which illustrate this point, thin coccal emulsions were combined with serum for long periods, and the mixtures required no centrifugalising before use.

Experiment VII.—Combination at 37° C. for 45 mins.

I. T. = 15 mins.

Temperature.	Cocci per leucocyte.
37° C.	9·6
18	8·0

Experiment VIII.—Combination at 37° C. for 2 hrs. 45 mins.

I. T. = 15 mins.

Temperature.	Cocci per leucocyte.
37° C.	15·0
18	14·5

Two further experiments similar to Experiment VIII gave ratios of 12·2 : 12·6 and 14·1 : 13·3 for the amount of phagocytosis at 37° C. to that at 18° C.

Experiment IX.—Comparison of the Effects of Combination at 37° C. and at 18° C.

Time of combination in each case, 1 hr. 40 mins. I. T. = 15 mins.

Combination at 37° C.		Combination at 18° C.	
Temperature.	Cocci.	Temperature.	Cocci.
37° C.	8·6	37° C.	4·9
18	8·5	18	6·1

It will be seen that after combination at 18° C. the amount of phagocytosis at 37° C. is even slightly exceeded by that at 18° C., though both fall very short of the corresponding figures obtained after combination at 37° C.

Experiment X.

Technique similar to that of Experiment IX. Temperatures of combination, 37° and 7° C. Time of combination, 3 hrs. 15 mins. Time of incubation, 20 mins.

Combination at 37° C.		Combination at 7° C.	
Temperature.	Cocci.	Temperature.	Cocci.
37° C.	23·0	37° C.	6·6
21	28·6	21	5·8
18	27·8	18	6·8
10	12·0	10	4·0

In each series, between the temperature range 37° C. to 18° C., the phagocytic values are practically identical, but the great differences between the absolute amounts in the two series afford a striking illustration of the superiority of combination at 37° C.

Experiment XI.—This experiment was designed to show the effect of varying the combination times at the two temperatures 37° C. and 18° C.

Samples for phagocytic purposes were removed from the serum-coccal mixtures every half hour and exposed to the action of the leucocytes.

Combination at 37° C.			Combination at 18° C.		
Temperature.	Combination time.	Cocci.	Temperature.	Combination time.	Cocci.
37° C.	30 mins.	5·0	37° C.	30 mins.	4·7
18	30 mins.	4·1	18	30 mins.	5·4
37	1 hr.	8·4	37	1 hr.	5·7
18	1 hr.	9·2	18	1 hr.	7·3
37	1 hr. 30 mins.	8·9	37	1 hr. 30 mins.	7·4
18	1 hr. 30 mins.	8·4	18	1 hr. 30 mins.	7·0
37	2 hrs.	10·7	37	2 hrs.	8·3
18	2 hrs.	10·5	18	2 hrs.	7·2

The pairs of phagocytic values in the various time intervals approximate very closely in the two series, showing that, under the conditions indicated the degree of phagocytosis is independent of the temperature within the range 37° C.—18° C. Further, when we compare the figures in the two series, those of the right hand series (combination at 18° C.) decidedly lag behind those of the left hand series (combination at 37° C.). The difference, however, is not nearly so marked as in a previous experiment, where the effects of combination at 37° C. and 7° C. were compared. One point is somewhat difficult to explain. In the case of combination at 18° C. it was found in an earlier experiment where thick emulsions were employed that the amount of phagocytosis at 18° C. fell considerably below that at 37° C. In the present experiment, where thin emulsions were employed for combining purposes, these values are equalised. The reason probably is that, although the rate of combination at 18° C. is slower than that at 37° C., prolonged contact of serum with cocci at the lower temperature (18° C.) effects a maximum absorption of opsonin by the cocci for that temperature, and, consequently, when phagocytosis takes place, the influence of temperature within the range 37° C.—18° C. is reduced to a minimum, just as in the case of combination at 37° C. In a paper by Bulloch and Atkin (1905) it is stated that the combination of the opsonin with the micro-organism takes place as readily at 0° C. as at 37° C. If this were so, it would be difficult to explain many of the experimental results detailed above. Dean (1905), on the contrary, observed that combination at 0° C. was much slower than at 37° C. The problem was again attacked in the following way :—

Experiment XII.—Emulsions of dead tubercle bacilli were incorporated with equal volumes of serum at the temperatures of 37° C. and 0° C. for a period of 30 minutes. The mixtures were then completely centrifugalised and the opsonic contents of the supernatant fluids towards fresh tubercle bacilli compared, fresh normal serum, in corresponding dilution, being employed as control.

	Bacilli per leucocyte.
Supernatant fluid after combination at 37° C.	3·4
” ” ” 0° C.	6·4
Fresh serum (diluted)	6·2

Experiment XIII.

Here the period of combination was prolonged for 11 hours.

	Bacilli per leucocyte.
Supernatant fluid after combination at 37° C.	2·5
” ” ” 0° C.	6·7
Fresh serum (diluted)	5·0

Experiment XIV.

Period of combination, 1 hr. 45 mins.

	Bacilli per leucocyte.
Supernatant fluid after combination at 37° C.	1·4
" " " 0° C.	5·1
Fresh serum...	7·1

These were the figures obtained after phagocytosis for 15 minutes. When phagocytosis was prolonged to 30 minutes the corresponding values obtained were 1·6, 11·3 and 10·9. The supernatant fluid, after combination at 37° C., had therefore been exhausted of its available opsonin after 15 minutes, whereas in the case of the second supernatant fluid (combination at 0° C.), the prolongation of the incubation period from 15 minutes to 30 minutes had the effect of more than doubling the phagocytic intake. Further, these fluids were tested against a fresh emulsion of staphylococci, when the following figures were obtained :—

0·9, 3·2, and 4·0 (control serum).

After combination at 37° C., therefore, the serum had lost nearly all its contents of tubercular and staphylococcal opsonins, whereas, after absorption at 0° C. little loss had occurred. It may be noted that incidentally the result tells against the specificity of the opsonin of normal serum.

There can be no doubt that absorption at 37° C. removes from a serum far more opsonin in the same time than absorption at 0° C. does. An attempt was made to test the degree of sensitisation of the centrifugalised bacilli after combination at 37° C. and 0° C. respectively. The supernatant fluids were removed and replaced by saline (twice repeated). When leucocytes were added to saline emulsions of the deposited bacilli, made up to the same volume and incubated at 37° C., little difference was found in the numbers taken up in the two cases. The technique is difficult and further experiments may have to be performed in this connexion, but if the phenomenon is constant it would show that at 37° C. the bacilli absorb more opsonin than is necessary for efficient sensitisation, while at 0° C. the bacilli remove just sufficient opsonin to prepare them for phagocytosis.

Summary and Conclusions.

1. When serum, cocci, and leucocytes are mixed directly and incubated at different temperatures, the number of cocci taken up increases more or less regularly with the temperature. By this method it has been shown that the phagocytic intake at 18° C. is only about one-fourth to one-fifth of that at 37° C.

2. This fall, at least within the temperature range 37° C.—18° C., is due to the diminished rate of combination of the serum with the coccus as the temperature falls.

3. When cocci which have previously been exposed to the action of serum, either at 37° C. or at 18° C., are put in contact with leucocytes, the intake is practically the same whether the phagocytosis takes place at 37° C. or at 18° C. The number taken up, however, after combination at 18° C. and more especially at 7° C., falls very short of the number taken up after combination at 37° C.

4. Experimental results, detailed above, lead one to assume that prolonged contact of a serum with cocci at a low temperature (18° C. or 7° C.) leads to a maximum absorption of opsonin by the cocci (corresponding to that temperature), so that the subsequent phagocytosis is identical whether it takes place at 37° C. or at 18° C.

5. Provided that cocci loaded with opsonin up to a certain maximum are presented to the leucocyte, the phagocytic energy of the latter is independent of the temperature within a wide range.

6. From the appearances on stained films it would seem that sensitised micro-organisms exposed to the action of leucocytes at very low temperatures tend to congregate near the periphery of the leucocytes, although little or no phagocytosis may take place. Hence, within a suitable temperature range, it may be presumed that the inclusion of sensitised micro-organisms by the leucocyte is a surface tension effect taking place between the coccus and the protoplasmic wall, amœboid energy playing only a subordinate part in the process.

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Nitrification in Acid Soils.

By A. D. Hall, M.A., N. H. J. Miller, Ph.D., and C. T. Gimingham.

(Communicated by H. E. Armstrong, Ph.D., LL.D., F.R.S. Received December 19, 1907,—Read February 6, 1908.)

(From the Lawes Agricultural Trust.)

Introductory.

Certain of the permanent grass plots at Rothamsted, which have been cut for hay and have received the same manurial treatment every year since 1856,* of late years have declined in yield, and the herbage has assumed an unhealthy condition. The plots affected are those to which nitrogen in the form of a mixture of ammonium chloride and sulphate is applied; the one receiving the highest amount of ammonium salts now carries a rank vegetation, consisting almost entirely of three species of grass —*Holcus lanatus*, *Alopecurus pratensis*, and *Arrenatherum avenaceum*— growing in coarse tufts with bare spaces between. The surface of these bare patches, which are spreading yearly, consists of a mat of peat-like decayed vegetation; a similar peaty formation is to be observed on the other plots receiving smaller amounts of ammonium salts.

In determining the nitrifying power of a number of Rothamsted soils,† Mr. S. F. Ashby observed that these grass soils failed to set up nitrification when small quantities were added to media suitable to the development of nitrates, indicating the comparative absence of the organisms causing nitrification. It was also noticed that the soil of these plots was distinctly acid, sufficiently so to redden blue litmus paper pressed against it in the moist state, a condition first observed by Voelcker‡ to be set up by the long-continued uses of ammonium salts on the arable soils of the farm of the Royal Agricultural Society at Woburn.

Inasmuch as the investigations of Warington and Winogradsky have shown that the process of nitrification can only go forward in the presence of a salifiable base and at once stops in an acid medium, it seemed possible that the observed decline in fertility of these plots might result from a cessation of nitrification due to the acid condition of the soil.

Though the soil of the grass plots is of the same origin and belongs to the same general type as that of the other Rothamsted fields—a stiff loam approaching clay and including a good many unworn flint stones—it differs in one essential respect from them, in that it contains but a small proportion of calcium carbonate, a substance which is usually present in the surface layer of the Rothamsted soil to the extent of from 2 to 5 per cent. It has been shown* that the calcium carbonate at Rothamsted only exists in the surface soil and is of artificial origin, being due to repeated "chalking" during the eighteenth century and earlier with chalk rock drawn from 10 to 12 feet below the surface. As the land in question was in grass before the practice had become general, it cannot have been often subjected to the chalking process, at any rate the surface soil now contains little calcium carbonate, 0·5 per cent. at the most, and the lower depths show less than 0·1 per cent. On Plot 11–1, which has received the largest amount of ammonium salts, the proportion is less, though the chalk has not been entirely removed, 0·16 per cent. being still present in the surface layer. But that the acidity of the grass soils is a consequence of the comparative absence of calcium carbonate there can be little doubt, since the soil of the arable fields at Rothamsted, which contains from 2 to 5 per cent. of calcium carbonate, is still neutral, though the same amounts of ammonium salts have been applied over even longer periods.

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* 'Phil. Trans.,' 1900, B, vol. 192, p. 139.

† 'Chem. Soc. Trans.,' 1905, vol. 85, p. 1158.

‡ 'Roy. Agric. Soc. Journ.,' 1904, vol. 64, p. 355.

To ascertain if the nitrifying organisms were present, small quantities of the soil were introduced into a suitable sterile culture medium containing an ammonium salt and other inorganic nutrients, together with a sufficiency of calcium or magnesium carbonate. Flasks containing 100 c.c. of such a solution were seeded with 0·2 to 0·5 gramme of soil, and were maintained at 30° C. with the usual precautions; after a month the liquids were tested for nitrates, nitrites, and ammonia. It is well known that nitrate or nitrite will be formed during such a period of incubation if any of the proper organisms have been introduced with the soil; as a rule, with normal soils the whole of the ammonia will be oxidised.

Samples were taken on six different occasions between October, 1904, and February, 1907, mostly from the surface soil only, but once down to 135 cm.; they were drawn from the continuously unmanured plot, from two plots receiving 400 lb. per acre per annum of ammonium salts, from one which received 600 lb. of ammonium salts, and from one plot to which sodium nitrate is applied in place of ammonium salts. Other samples were taken from the sections of these plots to which 2000 lb. per acre of quicklime had been applied in January, 1903.

On summarising the results, it appears that the soil of the unmanured plot from a depth of 10 to 15 cm. set up nitrification in seven out of eight trials, whilst with that from lower depths only failures were recorded, except in the case of two samples from 30 cm. The soil from Plot 4–2 (400 lb. ammonium salts) from a depth of 10 to 15 cm. only set up nitrification twice in nine trials, whilst that from lower depths proved active in six and inactive in four cases. The soil from Plot 9 [with the same ammonium salts], from a depth of 10 to 15 cm., failed to induce nitrification in 11 trials; with that from lower depths there were occasional successes. Soil from Plot 11–1, to which the largest quantity of ammonium salts is applied, never caused nitrification, except in the case of one sample from a depth of 45 cm., but the soil from Plot 14, which receives sodium nitrate instead of ammonium salts, induced a vigorous nitrification, except in the case of samples from depths of 105 and 135 cm., to which the nitrifying organisms are rarely likely to extend in heavy undisturbed grass land like that at Rothamsted.

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* Hall and Miller, 'Roy. Soc. Proc.,' B, 1905, vol. 77, p. 8.

The nitrifying organisms were more frequently found in the soil from the limed halves of the plots, though in the case of Plot 11–1 they were not detected in the upper layer.

These experiments indicated that the nitrifying organisms were present only sparingly in the soils which had received large amounts of ammonium salts, and that they were less abundant the more ammonium salts had been used, but that whenever the acidity of the soil had been reduced by the comparatively recent application of lime, nitrification was more frequently set up.

In order to verify these conclusions, two attempts were made to nitrify the soil itself in bulk. One or two kilogrammes of the moist soil, just as it came from the field, was spread out in a comparatively thin layer over water under a bell jar, so as to exclude dust and to maintain a moist atmosphere; after exposure in a dark part of the building during five weeks and two months respectively, the soils were rapidly dried and the amount of nitrates in them determined. The nitrates were also determined in another portion of the soil at starting, so as to ascertain the amount formed during the exposure under conditions favourable to nitrification. Of course, in sampling and manipulating such large quantities of material, it was impossible to prevent accidental introduction of the nitrifying organisms, but they would not affect the object of the experiment, which was to ascertain if the soils in their acid condition permit the nitrification process to go on. At the outset the soils were found to contain nitrates, but to a less extent than is usual. The quantities, however, varied from plot to plot with the amount of nitrogen supplied as manure; in one case, for example, the soil of Plot 11–1, receiving 600 lb. of ammonium salts, contained 12·44 parts of nitric nitrogen per million of soil, whilst the soil of Plot 9, which received 400 lb. of ammonium salts, contained 6·17 parts nitric nitrogen per million, and that of the unmanured Plot 3 contained only 1·78 parts. Such a relationship between the amount of nitrates in the soil and the amount of ammonia supplied to each plot indicates that the nitrates have been formed in the soil instead of being derived from some extraneous source common to all the plots; this conclusion is confirmed by the further behaviour of the soils during exposure, since in nearly all cases the nitrates were found to have increased. The five weeks' exposure was attended by an increase of nitrates in all the surface soils except in that of Plot 11–1, to which the largest amount of ammonium salts is applied; similar, though smaller, increases were shown by all the other samples, except those taken from the depth of 113 to 135 cm., soil from which depth had not been observed to contain nitrifying organisms in the previous series of experiments. The two months' exposure (surface soils only) produced similar effects, the increase in nitrates being small in the soils from Plots 9 and 11–1.

Although these results show that the nitrifying organisms cannot be wholly absent from the soil of the plots rendered acid by the use of ammonium salts, yet the quantity of nitrates produced during the exposure, favourable as were the conditions for nitrification, would not be sufficient to supply crops grown on these plots with the amount of nitrogen they usually remove from the land. During the five weeks' period, the nitrates in the soil of Plot 9 increased by two parts per million; in that of Plot 11–1 by 0·64 part per million; assuming that these optimum rates were maintained during a whole year, the total amount of nitrates produced would only amount to 50 lb. and 16 lb. respectively of nitrogen per acre. Yet, in 1904, Plot 9 produced 70·4 cwt. of hay, containing 94·6 lb. of nitrogen, and Plot 11–1 98·2 cwt. of hay, containing 131 lb. of nitrogen, all of which must have been derived from the soil, as no leguminous plants are present in the herbage.

From these figures it is difficult to resist the conclusion that though nitrification may not be entirely suspended in these acid soils, it is so far reduced that the plants cannot obtain in the form of nitrates all the nitrogen they take up from the soil, but must draw the larger portion directly from the ammonium salts supplied as manure. Müntz* and Mazé† have shown that maize, another gramineous plant, can derive its supply of nitrogen directly from ammonium salts, but attempts made by the authors to grow

* 'Comptes Rendus,' 1889, vol. 109, p. 646.

† 'Ann. de l'Inst. Pasteur,' 1900, vol. 14, p. 26.

some of the grasses characteristic of the plots under discussion in a medium containing unnitrified ammonium salts have, so far, failed.

The next step in the enquiry was, if possible, to determine the nature and amount of the acid present in the soil, in order to ascertain if it presented any special features which would explain the continuance of nitrification in its presence.

No satisfactory method exists of determining the acidity of a soil, owing to the sparing solubility of the humic acids and the difficulty of removing acid from so extensive a surface as that presented by soil particles. Extracting the soil with lime water, or dilute solutions of alkaline salts, gives results that are too high, because of the errors introduced by the presence of phosphates, and the interactions which may be set up with the zeolitic double silicates, ferric hydrate, and other soil constituents. Since we were chiefly concerned with the acidity of the soil water, we have used water only, accepting the fact that the results will be too low. When large quantities of the undried soil from the most acid plot, 11–1, were rapidly washed with small quantities of hot water, an extract was obtained which showed an acidity equivalent to 1·71 grammes of hydrogen per million of dry soil. After this extraction the soil still remained acid to litmus paper, even though the washing was repeated with large quantities of water, so that the acidity observed can only represent the easily soluble acids, leaving the greater part of the "humic" acids still in the soil residue. The chlorides and sulphates present in the water extract were equivalent to 1·91 and 3·15 grammes respectively of hydrogen per million of soil, the two together being equivalent to about three times the acidity. The manures supplied every year to this plot would add sulphates equivalent to 5·2 grammes and chlorides equivalent to 2·3 grammes of hydrogen per million of soil; consequently there was rather less than one year's stock of chlorides and sulphates in the top layer of soil at the time of sampling.

Under ordinary field conditions, the amount of water present in the soil will vary between 10 and 25 per cent., so that the easily soluble acids extracted by washing would give rise to an acidity of the soil water varying between one-sixtieth and one-hundred-and-fortieth of normal.

It is hardly necessary to enquire into the nature of the free acid present; the acid water extract contains humates, chlorides, and sulphates, hence it follows that such external factors as relative mass, temperature, and concentration, will determine which of them will be in the condition of a free acid. When the extract is concentrated, a certain amount of "humic" acid is thrown out of solution; when it is brought to complete dryness, hydrogen chloride is given off as gas. The real question is, whether the acid originates with the ammonium salts, or results from the attack of humic acid upon them. But free humic acid is not a normal product of decay in the soil; the unmanured plot, for example, is not acid; the "humus" of soil usually consists of calcium salts.* Further, the acidity of the grass plots under discussion increases with the amount of ammonium salts annually applied, so that it is to the ammonium salts we may look for the origin of the acid. Moreover, as the amount of freely soluble acid in the soil is of the same order of magnitude as the sulphuric and hydrochloric acids contained in one year's application of ammonium salts, we can suppose that it has been recently split off from them, but it does not accumulate from year to year, because it slowly acts upon the calcium humate present, forming the sparingly soluble free humic acid, and calcium sulphate and chloride which are removed in the drainage water. In confirmation of this opinion, it was found that a solution of ammonia would dissolve considerable quantities of humic acid from the soil of Plot 11–1, though in the case of normal soils it is necessary to break up the humates with hydrochloric acid before they will yield any humic acid to ammonia solution.

The next step in the enquiry was to find if any agency existed in the soil capable of splitting up ammonium salts so as to set free the acids therein. In a previous paper,† two of the authors have shown that no free acid results from purely chemical or physical interactions of ammonium salts and the soil constituents. Neither the double silicates nor the calcium humate in the soil exercise a selective absorption of the base to set free the acid, nor is there hydrolysis of the salt followed by adsorption or evaporation of the ammonia; the action between ammonium salts and the soil constituents is always in the nature of a double decomposition attended by no change in the neutrality of the medium. Being thus constrained to look for a biological explanation of the acidity in the grass soils, nutrient solutions were made up, containing, besides dextrose and the usual nutrient salts, ammonium sulphate or chloride as the only nitrogenous compound. These were rendered faintly acid and seeded either with the fresh soil or with a cold water extract from one of the acid grass plots. A vigorous crop of moulds and other fungi soon appeared, at the same time the liquid became markedly acid. The dominant organisms isolated in this way were forms of *Penicillium glaucum* and a mould allied to *Mucor*, and when pure cultivations of any of these species were seeded into solutions containing a carbohydrate and ammonium salts, the organisms were found to grow and to remove ammonia from the solution, which, at the same time, became acid, the acidity being sometimes greater and sometimes less than the equivalent of the ammonia withdrawn. The degree of acidity attained in this way lay between one-fiftieth and one-two-hundredth of normal; usually, growth stopped at about one-eightieth normal. It is significant that this is about the same degree of acidity as was found to prevail in the soil water of the acid grass plots. That the acidity is due to the attack of the organisms on the ammonium salts is seen from the fact that the culture solutions remain neutral when asparagin is substituted for the ammonium salts, or become alkaline when peptone is used as the source of nitrogen. Considering the abundance of these moulds in the peaty surface soil of the acid grass plots, and the rapidity with which they will work (*in vitro* the maximum acidity is attained in a week or ten days, just as the grass plots are most acid a few days after the application of the ammonium salts), the authors conclude that they are the active agents in producing the acidity of the soil of the grass plots in question. That the change in the ammonium salts takes this direction is due to the initial acid state of the soil; in such an acid medium the moulds have free play, while the nitrifying bacteria attacking ammonium salts in normal soils are checked or even suppressed. Doubtless the present acidity grew up gradually and locally as the original small stock of calcium carbonate in the soil became removed by the solvent action of the ammonium salts.

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* Hall and Gimingham, 'Chem. Soc. Trans.,' 1907, vol. 91, p. 677.

† Hall and Gimingham, *loc. cit.*

Having thus reached the conclusion, both by direct examination and consideration of origin, that the acidity observed in the soil is due simply to sulphuric and hydrochloric acids derived from the ammonium salts, how it is possible for nitrification still to go forward, since all investigators are agreed that the action is stopped as soon as the medium ceases to be neutral or faintly alkaline? To test the point further, water extracts were made from the soil of Plot 11–1, and concentrated until they represented the normal soil water, a little ammonium sulphate was added, and they were seeded with a strongly nitrifying extract from a garden soil and put to incubate. Check solutions were treated in the same way, but were first neutralised by the supply of calcium and magnesium carbonates. The nitrates were determined in portions of the soil extract at the outset and after three weeks' incubation, when it was found that no nitrification had taken place except in the neutralised solutions. Dilute cold water extracts from the acid soil also refused to nitrify except when neutralised.

When the extracts were not neutralised they were always invaded by moulds, which were especially abundant in the cold water extracts.

Thus there is found in the soil a degree of acidity which will inhibit nitrification *in vitro*; even a water extract of the soil will not nitrify, ye nitrification, though considerably reduced, does go on in the same soil under field conditions. The explanation is probably to be found in the want of uniformity in a solid material like soil; though the soil of Plot 11–1 is on the whole so acid, the analyses show that it still contains 0·16 per cent. of finely divided calcium carbonate, each of the grains of which would be a centre for nitrification, though the process might not be going on outside the region kept neutral by the calcium carbonate. Further, all chemical actions in a soil must be much modified and localised by having to take place in the thin film of water clinging by surface tension to the soil particles; diffusion would be very slow, so that interactions of a quite opposite nature might be taking place at the same time and within a very short distance.

This nitrification in acid soils is not unlike the case investigated by Chick,* who found that sewage nitrifies freely in passing through a coke bed, though the amount of organic matter dissolved in the sewage either entering or leaving the filter bed is sufficient to inhibit the nitrification of the liquid *in vitro.*

The authors conclude, then, that nitrification will continue in the Rothamsted soils as long as any particles of available calcium carbonate remain disseminated through them; the continuance of the present manurial treatment, however, must eventually reduce the soil to a uniformly acid condition, when nitrification may be expected to cease, just as happens in the case of peat or other naturally acid soils. The unhealthy condition of the plots may be set down to the fact that their acidity checks the work of the nitrifying and other bacteria and promotes instead the development of moulds. The moulds must compete with the grass for the nitrogen compounds supplied as manure, but whether they injure the crop in other ways is under further investigation.

EXPERIMENTAL.

A. *Soil and Manure.*

The following table shows the manurial treatment of the plots in question every year since 1856, together with the average yield of hay per acre and the nitrogen removed therein.

Plot.	Ammonium sulphate.	Ammonium chloride.	Super-phosphate.	Potassium sulphate.	Sodium sulphate.	Magnesium sulphate.	Average yield of hay.	Nitrogen per acre.
	lb.	lb.	cwt.	lb.	lb.	lb.	cwt.	lb.
3	—	—	—	—	—	—	21·6	33·9
4–2	200	200	3·5	—	—	—	35·7	66·1
9	200	200	3·5	500	100	100	54·4	76·8
11–1	300	300	3·5	500	100	100	66·8	107·2

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* 'Roy. Soc. Proc.,' 1906, B, vol. 77, p. 241.

To one-half of each of the plots, 2000 lb. per acre of ground quicklime was applied in January, 1903, the application being renewed in 1907.

Samples of soil taken in October, 1906, from the unmanured Plot 3, and from Plot 11–1, which receives the largest amount of ammonium salts, showed the following proportions of calcium carbonate, as calculated from the carbon dioxide evolved on treatment with hydrochloric acid.

Depth. inches.	Plot 3.	Plot 11–1.
0— 9	0·516	0·16*
10—18	0·206	0·151
19—27	0·092	0·106
28—36	0·087	0·141

B. *Examination of the Soil for the Nitrifying Organism.*

From 0·2 to 1 gramme of the soil under examination was added to 100 c.c. of a culture solution made up as follows:—

Ammonium sulphate..................	0·5 gramme per litre
Monopotassium phosphate	0·25 „
Magnesium sulphate (cryst.).........	0·15 „
Sodium chloride.........................	0·5 „
Ferrous sulphate	0·1 „

together with about 0·2 gramme of a mixture of sterilised magnesium and calcium carbonate. The solutions were in Erlenmeyer flasks, and had previously been sterilised in the usual way; they were then incubated for at least a month at a temperature of 30°, the contents being gently shaken two or three times during that period. At the end an examination of the clear liquid was made for nitrates with diphenylamine, for nitrites with metaphenylene-diamine, for ammonia with Nessler's reagent.

The soil samples were prepared as follows:—

In four series holes were dug in the field and sterile brass tubes 1 inch in diameter were forced into the sides, after breaking away some soil to expose a clean surface. The tubes were carried to the laboratory, the contents forced out into dishes and partly dried over strong sulphuric acid, suitable precautions being taken to avoid external contamination. The partly dried soil was then roughly powdered and stones removed by passing through a 1 mm. sieve, sieve and mortar being sterilised each time. By a suitable spoon a quantity found by trial to be approximately 0·2 gramme was introduced into the culture solution. For the last two series the sample of soil was extracted with an auger 2 inches in diameter having a slot in the side; at the selected depth in the slot a clean surface of soil was exposed and a small sample, approximately 0·5 gramme, was picked out with a sterile spatula and introduced into the culture flask, the operation being carried out in the field.

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* Since the above was written the authors have seen reason to suppose that some of the carbon dioxide, evolved on treating soils of this character with hydrochloric acid, comes from the organic matter. Several other processes agree to make the calcium carbonate in the surface layer amount only to 0·04 per cent.—*Note added March* 19, 1908.

Number of Times reacting after Four Weeks' Incubation.

Depth. cm.	Unlimed portion.		Limed portion.	
	Nitrate.	No nitrate nor nitrite.	Nitrate.	No nitrate nor nitrite.
Plot 3.—Unmanured.				
10—15	7	1	1	0
30	2	3	1	0
45	0	1		
75	0	1		
105	0	1		
135	0	1		
Plot 4–2.—Ammonium Salts and Superphosphate only.				
10—15	2	7	1	2
20—30	5	1	1	1
45	1	0	0	1
75	0	1	0	1
105	0	1	0	1
135	0	1	0	1
Plot 9.—400 lb. Ammonium Salts and Complete Minerals.				
10—15	0	11	3	4
20—30	2	3	2	1
50	1	0	1	0
70—75	2	0	0	1
105	0	1	0	1
135	0	1	0	1
Plot 11–1.—600 lb. Ammonium Salts and Complete Minerals.				
15	0	2	0	2
30	0	2	0	2
45	1	0	0	1
75	0	1	1	0
105	0	1	0	1
135	0	1	0	1
Plot 14.—550 lb. Sodium Nitrate and Complete Minerals.				
15	1	0		
30	1	0		
45	1	0		
75	1	0		
105	0	1		
135	0	1		

C. *The Nitrification of the Soils in Bulk.*

Large samples of the soil down to six depths of 9 inches were taken in October, 1906, and one portion of each was at once dried at a temperature of

70° to 80°, and the other placed in a thin layer under a bell jar over water. At the end of five weeks these second portions were similarly dried, and the two sets of samples, after removal of stones and reduction to a powder, were washed on a pressure filter with successive portions of water; the nitrates were determined in the extract by reduction and distillation of the ammonia. In the second trial the surface soil only, to a depth of 9 inches, was sampled in July, 1907; the exposure, made in the same way, was on this occasion continued for two months.

The following table shows the amount of nitric nitrogen per million of dry soil both before and after exposure, also the differences between the two sets of figures to show the amount of nitrates formed during the exposure.

Rothamsted Grass Soils. October, 1906.

Nitrogen as Nitric Acid per Million of Dry Soil.

Plot.		1st 9 inches.	2nd 9 inches.	3rd 9 inches.	4th 9 inches.	5th 9 inches.	6th 9 inches.
				In Soil before Exposure.			
3	Unlimed	1·06	—	0·54	—	2·19	1·33
4–2	Limed...	4·33	—	0·88	2·92	—	0·39
4–2	Unlimed	3·75	1·39	1·07	1·13	1·09	0·67
9	Limed...	2·25	1·75	1·35	0·79	1·08	2·00
9	Unlimed	2·38	1·11	0·90	1·20	0·47	0·58
11–1	Limed...	2·38	0·92	1·09	0·79	0·92	0·92
11–1	Unlimed	3·00	1·20	1·39	1·08	1·83	1·83
14	Unlimed	4·75	1·33	2·60	0·44	0·83	0·53
				In Soil after Exposure.			
3	Unlimed	1·41	0·97	0·67	1·08	0·96	0·63
4–2	Limed...	6·12	2·04	1·00	2·00	3·66	0·36
4–2	Unlimed	5·96	2·30	1·17	1·15	1·04	0·67
9	Limed...	2·70	2·54	1·30	1·32	1·25	1·49
9	Unlimed	3·42	1·15	0·77	1·39	1·14	0·79
11–1	Limed...	2·67	2·10	1·53	1·12	0·83	0·58
11–1	Unlimed	2·96	2·08	1·63	1·46	1·92	0·92
14	Unlimed	8·42	1·50	2·50	1·09	0·79	0·53
				Gain or Loss by Exposure.			
3	Unlimed	+0·35	—	+0·13	—	−1·23	−0·70
4–2	Limed...	+1·79	—	+0·12	−0·92	—	−0·03
4–2	Unlimed	+2·21	+0·91	+0·10	+0·02	−0·05	0
9	Limed...	+0·45	+0·79	−0·05	+0·53	+0·17	−0·51
9	Unlimed	+1·04	+0·04	−0·13	+0·19	+0·67	+0·21
11–1	Limed...	+0·29	+1·18	+0·44	+0·33	−0·09	−0·34
11–1	Unlimed	−0·04	+0·88	+0·24	+0·38	+0·09	−0·91
14	Unlimed	+3·67	+0·17	−0·10	+0·65	−0·04	0

July, 1907. First 9 inches of depth only.

Plot.	At starting.	After exposure.	Gain by exposure.
3	1·78	2·51	0·73
4–2	3·39	9·01	5·62
9	6·17	6·79	0·62
11–1	12·44	13·38	0·94
14	10·77	13·03	2·26

Two kilogrammes of an air-dried sample of the soil from Plot 11–1 were extracted with successive quantities of hot water and the extract concentrated to a volume of 500 c.c.; 50 c.c. portions of this were placed in the usual flasks for nitrification, 5 c.c. of a 1-per-cent. solution of ammonium sulphate was added, and each was seeded with 5 c.c. of a cold water extract from a garden soil in a good state of fertility. The object was to ascertain if the acidity of such a medium, which would represent the solution in the soil when it contains 25 per cent. of water, would be sufficient to inhibit nitrification after a fresh stock of the organisms had been supplied. The extraction of the dried soil had, however, not been very effective, and the initial acidity was only equivalent to 0·6 c.c. of *n*/10 alkali, or a little more than one-thousandth normal. In the check flasks the acidity was removed by the addition of a mixture of calcium or magnesium carbonates, and they were all incubated for three weeks at 30° C. Determinations were made of the nitrates before and after incubation, with the following results:—

50 c.c. Soil Extract + 5 c.c. Soil Extract + 5 c.c. 1-per-cent. $(NH_4)_2SO_4$.

		Nitrogen as nitrates. milligrammes.
1, 2, 3	No calcium or magnesium carbonate, incubated	0·95
4, 5, 6	With calcium and magnesium carbonates, incubated	2·38
7, 8	Not incubated	1·25

There was no formation of nitrates except when the acid had been neutralised by the calcium and magnesium carbonates. In 1, 2, and 3 weak growths of mycelium were visible, but none in the neutral extracts, 4, 5, 6.

In a further experiment fresh soil was brought from Plot 11–1 and extracted rapidly with cold water; the extract had a slight acidity, equal to 0·6 c.c. *n*/10 acid per 100 c.c. Portions of 50 c.c. were taken and 5 c.c. of 1-per-cent. ammonium sulphate were added to each; in some cases the extract alone was incubated for three weeks, with or without earthy carbonates; in others, 5 c.c. of a garden soil extract prepared as before was added to introduce the nitrification organisms. Nitrates were determined after a month's incubation, and in the checks before starting, with the following results:—

50 c.c. Soil Extract + 5 c.c. 1-per-cent. $(NH_4)_2SO_4$.

		Nitrogen as nitrates. milligrammes.
1 and 2	Not incubated	0·25
3 „ 4	No calcium and magnesium carbonate, incubated	0·23
5 „ 6	+ Calcium and magnesium carbonate, incubated	0·31
7 „ 8	+ 5 c.c. garden soil extract, no calcium and magnesium carbonates, incubated	0·39
9 „ 10	+ 5 c.c. garden soil extract, + calcium and magnesium carbonates, incubated	6·83

Again there was no formation of nitrates except when the acidity of the medium had been neutralised by the calcium and magnesium carbonates.

Flask 4 showed a heavy crop of moulds in fructification, 7 and 8 showed mycelium in the liquid, 10 one or two colonies of a bacterium on the surface.

D. *The Nature and Extent of the Acidity.*

Fresh soil to the depth of 9 inches was taken in March, 1907, a month after the application of the ammonium salts, and about 7 kilogrammes of the soil as it came from the field was extracted on a pressure filter with successive portions of hot water. No attempt was made to remove all the acid, as previous trials had shown this to be impossible with water alone; the soil after extraction still reddened blue litmus paper. The extract showed an acidity equivalent to 1·71 grammes hydrogen per million of dry soil. Sulphates and chlorides were also determined in the extract as follows:—

Chlorides equivalent to 1·91 grammes hydrogen per million soil.
Sulphates „ 3·15 „ „

When the soil was extracted with a fifth normal solution of sodium chloride, the acidity of the extract amounted to 30 grammes hydrogen per million of dry soil; substituting potassium nitrate for the sodium chloride, the acidity was equivalent to 42·5 grammes hydrogen per million of dry soil. In these two latter cases humic acid, insoluble in the water extract, was also being estimated.

Another sample was taken in November, 1907, and extracted in the same manner with the following results:—

Acidity equivalent to 1·02 grammes hydrogen per million soil.
Chlorides „ 0·35 „ „
Sulphates „ 1·60 „ „

At this latter date a sample was taken from Plot 9 and gave the following results:—

Acidity equivalent to 0·36 grammes hydrogen per million soil.
Chlorides „ 0·47 „ „
Sulphates „ 1·69 „ „

In this case some of the humic acids dissolved in the original extract were thrown out during the evaporation of the extract, hence the acidity measured is a little low. The acidity of the soil persists after drying in the air, but is measurably reduced when the soil is dried in the steam oven.

If these measured acidities are calculated on the proportion of water usually found in the soil, which will vary between 10 and 25 per cent. of its dry weight, the following results are obtained:—

Approximate Acidity of Soil-water. Fractions of normal.

	When soil contains 10 per cent. water.	When soil contains 25 per cent. water.
Plot 11–1, March, 1907	1/60	1/150
Plot 11–1, November, 1907...	1/100	1/250
Plot 9, November, 1907	1/300	1/700

To ascertain if the acid soils contain any free humic acid, 10 grammes of the soil of Plot 11–1 were extracted with 500 c.c. of 4-per-cent. ammonia solution, and the amount of organic matter going into solution was determined. On a second 10 grammes the usual process of determining "humus" was followed; the soil was treated with hydrochloric acid, and the acid washed away before extracting with the ammonia solution. Parallel experiments were made with a prairie soil rich in humus but neutral, since it contained 3·5 per cent. of calcium carbonate. The following results were obtained:—

	Organic matter soluble in ammonia.	
	Without acid treatment.	After preliminary acid treatment.
Plot 11–1	3·54 per cent.	4·51 per cent.
Prairie 0–4	Nil	4·65 "

The prairie soil yielded no humic acid to ammonia, unless the humates it contained had first been decomposed by hydrochloric acid, whereas the soil of Plot 11–1 contained 3·54 per cent. of humic acid immediately soluble in ammonia.

E. *Liberation of Acid from Ammonium Salts.*

To ascertain if any of the organisms in the soil of these acid plots were capable of setting free ammonia from its salts, small Erlenmeyer flasks, containing a solution made up of 2 grammes ammonium chloride, 0·5 gramme monopotassium phosphate, 0·25 gramme magnesium sulphate, 1 gramme sodium chloride, and 10 grammes of glucose per litre, were sterilised, inoculated with the soil or soil extract, and incubated at 21° C. After seven days a vigorous growth of various moulds was observed, and the medium was found to be distinctly acid. In mixed cultures of this kind the acidity rose from about $n/166$ to $n/80$ in a week, and then remained constant. Sub-cultures were made and the organisms isolated in the usual way: the dominant species were forms of *Penicillium glaucum* and a *Mucor* (?), which differs in several respects from those already known, and does not appear to have been described.* Samples taken from Plots 11–1, 9, and 4–2 at different times during the spring and summer showed the same dominance of *Penicillium* and the *Mucor*. Various other species of

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* This mould has since been identified by Professor A. F. Blakeslee as *Zygorhynchus Moelleri*, Vuill.—*Note added March* 19, 1908.

Mucor, Trichoderma, and *Acrostalagmus* have also been observed, but the description of the fungus flora present in the soil of these plots will be given elsewhere. The essential point is that both the dominant species, *Penicillium* and the *Mucor,* and several of the others examined, when grown into a nutrient medium containing a carbohydrate and an ammonium salt, draw the nitrogen they require from the ammonia and leave the acid in a free state. This production of acid by *Penicillium* growing in a medium containing an ammonium salt has already been observed.* The following experiments will serve to illustrate the extent to which the action takes place:—

Experiments with Mucor sp. ?—Flasks were made up with 60 c.c. of a nutrient solution containing varying amounts of ammonium chloride and cane-sugar as under; the flasks were incubated at 21° C., and the acidity determined after 34 days, with the results set out in the last columns:—

	NH_4Cl. gramme.	Cane-sugar. grammes.	Gain of acidity as c.c. $n/10$ acid.	Percentage of ammonium chloride converted.
A 1	0·6	1·2	11·6	13·9
A 2	0·6	0·6	8·0	7·2
B 1	0·3	1·2	8·1	14·5
B 2	0·3	0·6	6·9	12·3
C 1	0·06	1·2	9·1	81·7
C 2	0·06	0·6	8·5	75·0

The highest acidity observed at the end was equivalent to about a $n/50$ solution of free acid.

When a small quantity of asparagine was also added to the nutrient solutions the increase of acidity was negligible; the organism satisfied its requirements for nitrogen from the asparagine in preference to the ammonium chloride, which, in consequence, remained undecomposed.

Experiments with Penicillium.—One of the forms of *Penicillium* was seeded into a solution containing 1 per cent. glucose, 0·25 per cent. ammonium chloride, and the usual nutrient salts. Growth was fairly vigorous and was stopped after 14 days, when the yellow solution was filtered off and tested for acidity and ammonia, showing the following results for 100 c.c. of the original solution:—

	Acidity as c.c. $n/10$.	Nitrogen as ammonia.	Ammonia removed.
Check	0	0·063	
A	5·5	0·059	0·004 = 2·8 c.c. $n/10$ acid
B	5·2	0·058	0·005 = 3·5 „

The acidity in this case has been increased, though not to the extent found with the *Mucor*; at the same time, ammoniacal nitrogen has been withdrawn from solution in amounts accounting for rather more than half of the observed acidity.

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* Kohn and Czapek, 'Beitr. Chem. Physiol. Path.,' 1906, vol. 8, p. 302.

Another set of observations made by Dr. H. B. Hutchinson gave the following results:—

Acidity produced and ammonia withdrawn from solution containing 1 per cent. dextrose and 0·2 per cent. ammonium sulphate, during 21 days' growth of various moulds isolated from Plot 11–1.

Cubic centimetres of Tenth Normal Acid per 100 c.c. Culture Medium.

Organism.	Acidity.	Acid equivalent of ammonia withdrawn.
Mucor sp. ?	6·0	11·1
Mucor I	4·1	5·6
Mucor II	5·44	9·6
Penicillium	7·5	12·4
Acrostalagmus	10·1	11·9
Trichoderma I	11·3	13·9
Trichoderma II	12·54	13·6

In these cases there was always a greater amount of ammonia withdrawn from solution than was equivalent to the acidity measured, which, as before, amounts to a concentration of from $n/200$ to $n/70$ in the final solution. It is further significant that the degree of acidity developed in the culture solutions is of about the same order, between fiftieth and two-hundredth normal, as that which was found to exist in the soil water of the field plots.

Summary.

In the soil of certain of the permanent grass plots at Rothamsted, which is distinctly acid in consequence of the long-continued use of ammonium chloride and sulphate as manure, nitrification is greatly reduced, and the nitrifying bacteria are only found sparingly. In bulk, nitrification still goes on slowly, despite the acidity of the soil. Water extracts of the soil will not permit of nitrification unless they are previously neutralised. The amount of nitrate produced would not be sufficient for the nitrogen taken up by the crop, which must, in the main, utilise the ammonium salts without previous change. The acidity is chiefly due to sparingly soluble "humic" acids; free hydrochloric and sulphuric acids are also present, because the soil extract contains soluble acid in quantities comparable to the amount of chlorides and sulphates also present, and to the ammonium sulphate and chloride annually supplied as manure.

The acidity is not brought about by purely chemical or physical actions of the soil upon the ammonium salts, but by various micro-fungi which are able to remove ammonia from a solution of its salts and set free the acids with which it was combined, the acidity attained in this way being equivalent to that of the soil water on the acid plots.

The authors attribute the continuance of the nitrification in these soils to the irregular distribution of the materials composing them; though acid as a whole, they still contain some calcium carbonate, each of the particles of which forms a centre for the nitrification process. The decline in fertility of the acid plots may be attributed to the repression of the normal bacterial activities of the soil and the encouragement of the growth of moulds.

The Origin and Destiny of Cholesterol in the Animal Organism.
Part I.—*On the so-called Hippocoprosterol.*

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(Communicated by Dr. A. D. Waller, F.R.S. Received December 20, 1907,—Read January 23, 1908.)

(From the Physiological Laboratory of the University of London.)

Introductory.

Since the discovery of cholesterol by Conradi in 1775, and its analysis by Chevreul in 1815, it has been found to be very widely distributed in the animal and in isomeric forms in the vegetable kingdom. It is found in small quantities in all protoplasmic structures, in blood, bile, sebum, and similar oily excretions of the skin, and is an especially abundant constituent of the white substance of brain and of the medullary sheath of nerve. But, although a considerable amount of work has been done, we have little or no definite knowledge of its physiological functions, and it is only in very recent times that a small glimmering of light has been thrown on its chemical constitution.

In 1862, Austin Flint* published a series of experiments by which he attempted to show that cholesterol is always more abundant in the blood coming from the brain than in the blood of the general arterial system, or in the venous blood from other parts; that its quantity is hardly appreciable in venous blood from the paralysed side in hemiplegia, and that it is separated from the blood by the liver. He also stated that in cases of serious structural disease of the liver, accompanied by symptoms pointing to blood poisoning, cholesterol accumulates in the blood, constituting a condition which he named cholesteræmia. From his experiments he came to the conclusion that cholesterol is a product of the metabolism of nervous tissues, that it is carried from the brain by means of the blood and excreted by the liver through the bile, and, finally, that "we know of no function which it has to perform in the economy, any more than urea or any other of the excrementitious principles of the urine." Flint's methods of analysis were, however, open to grave objection, and he draws sweeping conclusions from differences so slight that had his method of estimating cholesterol been capable of considerable accuracy, we should have hesitated to attribute much significance to the figures. Flint also observed* that the cholesterol of the bile undergoes a modification in the intestine, and is found in the fæces as "stercorine." Some support was lent to Flint's views by the experiments of Picot† in 1872 and Koloman Müller‡ in 1873. Picot reported a fatal case of "grave jaundice," in which he found a great increase in the proportion of cholesterol in the blood; and Müller injected into the veins of dogs 2·16 fluid ounces of a solution containing 69 grains of cholesterol, made by rubbing cholesterol with glycerine and mixing the mass with soap and water. In five experiments of this kind, he produced a complete representation of the phenomena of "grave jaundice."

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* "Experimental Researches into a new Excretory Function of the Liver," 'American Journal of Medical Sciences,' Philadelphia, 1862, new series, vol. 44, and "Recherches Expérimentales sur une Nouvelle Fonction du Foie," Paris, 1868.

How far Flint's views obtained general credence we do not know, for he is rarely quoted in physiological text-books, and we are unaware of any other extensive series of experiments on the subject; but B. Moore, in Schäfer's 'Text-book of Physiology' (1898), states that "according to Hoppe-Seyler cholesterol is a cleavage product, constantly formed in the metabolic changes in the living cell; and for this reason it is that cholesterol is invariably found as a chemical constituent of both animal and vegetable cells. Cholesterol does not easily undergo decomposition in the animal organism when once formed, and is principally excreted in the higher animals in the bile. It is formed in increasing quantity in tissue which is undergoing pathological change . . . . It is probably formed most in the metabolism of nerve tissue, taken up by the liver cells from the blood, and passed as an excretion into the bile ducts. Cholesterol is purely an excretion, and is not reabsorbed, but passes out of the body with the fæces." Some more recent observers appear to hold the view that the cholesterol of the bile is formed either in the liver cells or in the cells lining the bile passages or both, but the evidence for this is not particularly convincing. That some cholesterol is formed somewhere within the liver, and not merely excreted by it, seems to be shown by an experiment of Jankau,* performed in Naunyn's laboratory. He injected cholesterol into dogs, and also gave it in their food, and ascertained that it had been absorbed; but he failed to find any increase of cholesterol in the liver tissue or in the bile. The analyses of the liver and the bile published by Kausch† from the same laboratory show no relationship between the amount of cholesterol in the gland and in its secretion. Thomas,‡ also working under Naunyn's direction, found that there is no relationship between the amount of cholesterol excreted and the kind of food taken. When, however, the dog under observation suffered from catarrh of the biliary passages, there was a marked increase in the cholesterol of the bile.

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* *Ibid.*

† 'Journal de l'Anatomie,' Paris, 1872, vol. 8.

‡ "Ueber Cholesterämie," 'Archiv für Experimentelle Pathologie und Pharmakologie,' Leipzig, 1873, vol. 1, p. 213.

[Goodman,§ on the other hand, in an experiment on a dog with a permanent fistula, observed that the quantity of cholesterol found in the bile varied considerably with the nature of the diet, but that the amount of cholesterol in the food taken was without influence. Thus white of egg and calves' brain were equally efficacious in increasing the output of cholesterol, although the brain contains some 2 per cent. of its weight of cholesterol and the egg albumin practically none. He found, too, that intravenous injection of cholesterol did not increase the output in the bile, the result being in agreement with that of Jankau. Pribram|| also has shown that in the case of rabbits which have been fed with cholesterol for some days and then killed, there is a decided rise in the cholesterol content of the blood, which, in consequence, exhibits an increased power of resistance to the hæmolytic effect of saponin.]¶

From these experiments, and from the fact that cholesterol is always found where cells are disintegrating, Naunyn strongly supports the view that cholesterol is produced, not in the liver cells, but from the cells of the passages,** and that it is a product of disintegration of their protoplasm. More recently, V. Harley and W. Barratt,†† in a series of experiments on the

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* 'Cholelithiasis,' transl. by A. E. Garrod, New Syd. Soc., 1896.

† Dissertation, Strassburg, 1891.

‡ 'Cholelithiasis, *ibid.*

§ 'Hofmeister's Beiträge,' vol. 9, p. 91.

|| 'Biochem. Zeit.,' 1906, vol. 1, p. 413.

¶ The passage within square brackets is added as the paper passes through the press.

** *Cf.* also Doyon and Dufort, 'C. R. Soc. Biologie,' 1896, vol. 10 (3), p. 487.

†† "An Experimental Enquiry into the Formation of Gall Stones," V. Harley and W. Barratt, 'Journ. Physiol.,' vol. 29, p. 341, 1903.

effect of introducing gall stones into the gall bladders of dogs, have shown that when the gall bladder is healthy the gall stones tend to disappear, while, on the other hand, when cholecystitis is present they remained unchanged.

In his Text-book of Physiology, Schäfer has also suggested that the constant presence of lecithin and cholesterin in the bile may well be associated with the destruction of the red corpuscles, which contain relatively considerable amounts of these substances.

It will be seen from the foregoing sketch that we know little or nothing definite as to the functions of cholesterol, and we certainly know nothing whatever of the breaking up of cholesterol in the animal body.

From the very general occurrence of cholesterol and its frequent association with lecithin, we cannot but think that it must play an important part in the cell economy, and must not be considered merely as a waste product. In this connection the experiments of Flexner, Noguchi,* Preston Kyes,† Abderhalden and Le Count,‡ on hæmolysis produced by cobra poison in the presence of lecithin and the inhibitory effect of cholesterin appear to us very significant.

Whatever may be the value of Flint's views on the cholesterol problem, he was correct in his statement that cholesterol is found in human fæces in the modified form of "stercorine."

This body was rediscovered in 1896, by Bondzynski§—who gave it the name of coprosterol—and was thoroughly investigated by Bondzynski and v. Humnicki.|| It crystallises in long slender needles melting at 95° to 96° C. and behaves chemically as a saturated alcohol; it is dextrorotatory and gives colour reactions similar to those of cholesterol. Its formula, $C_{27}H_{48}O$, was confirmed by the analyses of a large number of derivatives, and its discoverers regarded it as a dihydrocholesterol formed by bacterial reduction in the intestine. They fed a man with cholesterol and found that it was excreted mainly as coprosterol, and, later, Müller¶ proved that on a milk diet, in which

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* "Snake Venom in Relation to Hæmolysis, Bacteriolysis, and Toxicity," 'Journ. of Experim. Med.,' vol. 6, No. 3, 1902.

† "Ueber die Wirkungsweise des Kobragiftes," 'Beit. klin. Wochenschr.,' Nos. 38 and 39, 1902; also "Lecithin und Schlangengift," 'Zeit. f. physiol. Chemie,' vol. 41, p. 273, 1904; also "Zur Kentniss der Kobragift aktivierenden Substancen," 'Berl. klin. Wochenschr.,' Nos. 2—4, 1903.

‡ "Die Beziehungen zwischen Cholesterin, Lecithin, Kobragift, Tetanuslaxin, Saponin und Solanin," 'Zeitschr. f. experim. Path. u. Therap.,' vol. 2, p. 199, 1905.

§ "Cholesterol of Human Fæces," 'Ber. der Deut. Chem. Ges.,' 1896, vol. 29, p. 476.

|| "The Fate of Cholesterol in the Animal Organism," 'Zeit. Physiol. Chem.,' 1896, vol. 22, p. 396.

¶ "Reduction of Cholesterol to Coprosterol in the Human Intestine," 'Zeit. physiol. Chem.,' 1900, vol. 29, pp. 129—135.

putrefactive changes in the intestine are reduced to a minimum, the cholesterol of the body is excreted unchanged. The reduction of cholesterol in the intestine thus seems established in the case of man, and Bondzynski and Humnicki, continuing, examined the fæces of the dog and the horse.* In the case of the dog, they stated that the cholesterol of the bile was excreted unchanged, but in that of the horse intestinal reduction went much further than in man, and a cholesterol-like body, $C_{27}H_{54 \text{ or } 56}O$, was found, crystallising in microscopic needles melting at 74° to 75° C.

This hippocoprosterol, as it was called, was further examined in 1905 by Wilenko.† The results of his work go to prove that the excrement of the horse contains two isomeric bodies, $C_{27}H_{52 \text{ or } 54}O$, one readily soluble, the other much less soluble, in 97 per cent. alcohol. These he designated as α and β-hippocoprosterol respectively. The α body crystallises in minute rhombic tables similar to cholesterol crystals. When dry it forms silky scales which are as soft as wax and melt at 66° to 67° C. The β isomer appears to be identical with Bondzynski and Humnicki's hippocoprosterol, though Wilenko gives its melting point as 56° instead of 74°.

In order to throw some further light on the origin and functions of cholesterol in the animal economy, it appeared to us in the first instance essential, more especially considering the discrepancies and scantiness of the work of previous observers, to make a comparative study of the forms in which cholesterol is found in the fæces of different animals and to determine to what extent the substances thus excreted are dependent upon the food taken. In the present paper we give an account of our experiments on the fæces of herbivorous animals, those examined being the horse, cow, sheep, and rabbit.

Method of Experiment.

The material was obtained from grass-fed animals (Hampshire) and was sometimes dried directly in the water oven, but generally spread out in thin layers and allowed to dry in the air. In order to deal effectively with such a light, bulky substance, we employed large metal extractors capable of holding 2 to 5 kilogrammes of material. They were made on the Soxhlet pattern, fitted with long metal condensers, and the ether vessel was enclosed in an air chamber warmed with incandescent electric lamps. The dried material was extracted usually for five to six days with ether, the dark green solution obtained diluted, if necessary, with more ether and at once saponified with sodium ethylate in alcoholic solution according to the method of Kossel and Obermuller: care had, however, to be taken not to allow the alcohol added to amount to more than 1/10 to 1/12 of the volume of the ether, lest any of the hippocoprosterin should be precipitated along with the soaps. The mixture was well shaken, allowed to stand overnight, and the soaps filtered off and washed with ether. The filtrate was then shaken, first with an equal bulk of water to remove alkali and alcohol, and then with water containing potash to remove the last traces of soap. The ether solution was finally separated off, dried, and evaporated.

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* *Ibid.*

† "Hippocoprosterol," M. Gittelmacher Wilenko, 'Bull. International de l'Académie des Sciences de Cracovie,' No. 1, Jan., 1906, p. 20.

The crude extract was dark red, liquid at 100° C., and had a pungent smell like that of wood spirit. It dissolved in boiling alcohol (with the exception of a small residue soluble in benzene), giving a deep red solution which could be partially decolorised by treatment with charcoal. The charcoal, however, subsequently required repeated extraction with alcohol, as it obstinately retained the substance. The pale yellow filtrate, on cooling, set to a bulky gelatinous mass which was difficult to filter and left a solid which, on drying, shrank up to light flakes of impure hippocoprosterol. This was dissolved in ether and precipitated by alcohol, the process repeated, if necessary, and the nearly white material dissolved in ether and allowed to crystallise by slow evaporation.

The alcoholic mother liquors obtained in the above processes were subjected to further crystallisation till only oily matter remained.

Examination of Horse Excrement.

The hippocoprosterol obtained, as above, was purified by crystallisation from benzene or ethyl acetate, and finally from ether. Sometimes it was necessary to use charcoal again before the last traces of colour could be removed, but often this could be dispensed with. The yield was 0·2 per cent. of the dry fæces.

Hippocoprosterol is a light, white substance, which may be powdered, but cakes together under slight pressure. It dissolves at the boiling temperature in all the usual solvents (except water), but comes out again almost completely in the cold. Thus its solubility in benzene at 16° C. is only 0·32 part per 100 parts of solvent. From ethyl and methyl alcohol, ethyl acetate, glacial acetic acid and acetic anhydride, it comes out as a white jelly; from ether, petrol ether, benzene and chloroform in a more powdery form. From strong solutions it crystallises in microscopic needles grouped in stars, rosettes, and comet-shaped clusters, but on slow evaporation these clusters may be obtained 1 to 2 mm. in diameter. If a dilute ethereal solution be allowed to evaporate spontaneously, large transparent masses of crystals are obtained, consisting of fan-shaped groups of needles springing from a common base. It melts at 78°·5 to 79°·5 C., and solidifies at 77°, a characteristic property. It is optically inactive, and does not give the cholesterol colour reactions. These observations are at variance with the statements of Bondzynski and Humnicki, who attribute to hippocoprosterol a slight dextrorotation and a green colour reaction in a modified Salkowski test, but seeing that their product melted at 74° to 75° C., it probably contained some impurity which produced these effects. Wilenko's β-hippocoprosterol, which is undoubtedly identical with ours, he describes as melting at 56°, a discrepancy we are unable to understand, since on elaborate purification of our product we failed to obtain any alteration in the melting point. Hippocoprosterol does not absorb bromine in carbon bisulphide solution. Dry bromine in a sealed tube at 100° C. is without action, but at 170° C. various substitution products are obtained.

For analysis the substance was dried *in vacuo* at the ordinary temperature, and was burnt in a copper boat filled with coarse copper oxide.

I. 0·1936 gave 0·5823 CO_2 and 0·2473 H_2O.
II. 0·1447 „ 0·4352 CO_2 „ 0·1738 H_2O.

	Found.		Calculated for	
	I.	II.	$C_{27}H_{54}O$.	$C_{27}H_{56}O$.
C	82·03	82·03	82·14	81·73
H	14·13	14·11	13·80	14·24

A molecular weight determination, carried out by the cryoscopic method, showed that it possesses the simple formula:—

0·8321 gramme in 13·36 grammes of naphthalene (constant = 70) gave a depression of 1°·157.

Molecular weight found, 377. $C_{27}H_{54}O$ requires 396.

Hippocoprosterol Acetate.—One part of hippocoprosterol was mixed with two parts of fused sodium acetate and five to six parts of acetic anhydride, and heated to boiling for half an hour. The product was poured into water, and the precipitated acetate dissolved in acetic ether. It came out from this in flocks, which consisted of microscopic needles resembling the original substance. The yield was almost quantitative.

The acetate is moderately soluble in alcohol and acetic ether, easily in ether, benzene and petrol. From the latter it was obtained in shining flakes, which were sticky, caking together under the least pressure. Melting point 61° to 62° C.

On analysis, the following figures were obtained:—

I. 0·1719 gave 0·5035 CO_2 and 0·2061 H_2O.
II. 0·1831 „ 0·5333 CO_2 „ 0·2181 H_2O.

	Found.		Calculated for	
	I.	II.	$C_{27}H_{53}O.CO.CH_3$.	$C_{27}H_{35}O.CO.CH_3$.
C	79·88	79·43	79·73	79·37
H	13·32	13·24	12·93	13·33

On saponification of the acetate with sodium ethylate, hippocoprosterol was obtained, melting at 79° C.

Hippocoprosterol Benzoate.—The hippocoprosterol was mixed with an equal weight of benzoic anhydride, and heated to 160° for two hours in an open vessel. The product was boiled with alcohol, and on cooling the benzoate separated in thick clots. These were recrystallised several times from petrol, and finally from ethyl acetate. Hippocoprosterol benzoate crystallises in microscopic needles, which in mass appear as sticky lumps. It is difficultly soluble in alcohol and ethyl acetate (but more so than the mother substance), easily in ether, petrol, and benzene. The melting point is 58°·5 to 59°·5 C. In concentrated ether solution it proved optically inactive, and on analysis gave the following figures:—

0·1799 gave 0·5395 CO_2 and 0·1975 H_2O.

		Calculated for	
	Found.	$C_{27}H_{53}O.CO.C_6H_5$.	$C_{27}H_{55}O.CO.C_6H_5$.
C	81·79	81·85	81·52
H	12·20	11·73	12·09

Two grammes of the benzoate were saponified with sodium ethylate and yielded hippocoprosterol, melting at 79°.

*Hippocoprosterol Cinnamate.**—Five parts of hippocoprosterol were mixed with three parts of cinnamyl chloride and heated to 140° for one hour. The product was boiled out with alcohol, and the solution on cooling deposited the cinnamate in masses of minute needle-shaped crystals which, when dry, appeared sticky, caking together under pressure. It is very soluble in benzene, moderately in acetic ether and petrol, and difficultly in alcohol. The melting point is 62° C. On analysis the following figures were obtained:—

0·2040 gave 0·6143 CO_2 and 0·2132 H_2O.

		Calculated for	
	Found.	$C_{27}H_{53}O.CO.C_8H_7$.	$C_{27}H_{55}O.CO.C_8H_7$.
C	82·17	82·37	82·05
H	11·61	11·53	11·81

* This paragraph is added as the paper passes through the press.

Hippocoprosterol behaves, therefore, as a saturated alcohol. The hydroxyl group, however, is not readily replaceable by chlorine. When ground up with phosphorus pentachloride in the cold, no action takes place, though on adding petrol and boiling some hydrochloric acid is evolved, but we have not succeeded in preparing the chloride, either by this method or by the use of thionyl chloride. Various substances appear to be formed, which we are at present investigating.

Examination of the Alcoholic Mother Liquors.

The residues remaining after separation of the hippocoprosterol were subjected to a careful examination, first with the object of isolating the α-hippocoprosterol (m.p. 66° to 67°) described by Wilenko, and secondly to discover whether any of the cholesterol of the bile was present.

As an example, 2 kilogrammes of dry dung obtained in the summer yielded 3·65 grammes or 0·18 per cent. of hippocoprosterol, and 8·1 or 0·4 per cent. of a dark red buttery residue. The latter was dissolved in alcohol and allowed to evaporate. The first crops of material obtained gave melting points varying between 65° and 70°, but small quantities of pure hippocoprosterol could always be obtained from these. The second crop (0·2 gramme) gave a body which dissolved easily in all solvents, except dilute alcohol, from which it crystallised readily in microscopic hexagonal plates. It melted at 136° to 137°, absorbed bromine in carbon bisulphide solution, and gave the Salkowski and Liebermann colour tests. It thus agreed closely with the sitosterol of Burian.* The acetate of the body, however, did not confirm this. It was made in the usual way with acetic anhydride and sodium acetate, and came out of dilute alcohol in brilliant glistening leaves consisting of six-sided plates. In all its properties, however, it agreed with the original substance, except that it was less soluble in absolute alcohol. It gave a constant melting point of 136°, which could not be altered by repeated crystallisation. Sitosterol acetate melts at 127°·5. The third crop (0·1 gramme) was white, and after several crystallisations from 80-per-cent. alcohol appeared under the microscope as long thin plates like sword blades. These melted sharply at 161° to 162° and gave Liebermann's colour test. To identify it if possible with the caulosterine obtained by Schulze and Barbieri† from the shoots of the yellow lupin (m.p. 158°), it was heated with benzoic anhydride, but no crystalline benzoate could be obtained. The concentrated alcoholic filtrate still showed traces of solid matter, but this could not be obtained free from oil. The alcohol was accordingly evaporated off, and the buttery residue, which dissolved very easily in most solvents, was treated with 75-per-cent. alcohol, in which it was more difficultly soluble. From this a white solid body was obtained in small quantity (less than 1 per cent.), which gave Liebermann's test and crystallised from dilute alcohol in the sword blade plates described above. From ether and petrol it dried up to rosettes of needles which softened at 145° and melted clear at 154° to 155°. This melting point could not be raised by repeated crystallisation. To discover whether it was identical with Tanret's ergosterol,* it was converted into the acetate by heating with acetic anhydride and sodium acetate. The product, which was very soluble in petrol, crystallised from dilute alcohol in microscopic rectangular plates. It, however, melted at 78° to 80° C., whereas Tanret's acetate melted at 169° to 176°.

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* From germinating wheat, 'Monatshefte f. Chemie,' 1897, vol. 18, p. 551.

† 'Journ. Prak. Chem.,' (2), vol. 25, p. 159.

After separation of the solid bodies as above, the alcoholic solution leaves, on evaporation, a very dark red thick oil, which, in the case of the horse, has not been further investigated. It is important, however, to emphasise that, although we have extracted some 15 kilogrammes of dried fæces, we have not been able to obtain either the α-hippocoprosterol of Wilenko, or to detect any trace of cholesterol, microscopically or otherwise. Instead, we have obtained in minute quantity high melting bodies, which, so far as they could be examined, appeared to belong to the phytosterol, or vegetable cholesterol, group.

Examination of the Excrement of the Cow and Sheep.

The material was obtained from grass-fed animals, and was extracted and worked up as before. The chief product was in each case a body identical in all its properties with the hippocoprosterol described above. The yield amounted to 0·15 per cent. of the dry dung in the case of the cow, and 0·3 per cent. in that of the sheep. The identity was proved by the crystalline form, solubility, melting points, mixed melting points, and analysis.

I.	Cow	0·2383	gave	0·7168 CO_2	and	0·3025 H_2O.
II.	Sheep	0·1578	„	0·4780 CO_2	„	0·2028 H_2O.

	Found.		Calculated for
	I.	II.	$C_{27}H_{54}O$.
C	82·02	82·61	82·14
H	14·10	14·28	13·80

The cow also gave about 0·3 per cent. of a dark red oil similar to that of the horse, while the sheep gave 0·4 per cent. of a yellow vaseline-like substance smelling strongly of hay.

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* The ergosterol described by Tanret ('Annales de Chim. et de Physique,' series 6, vol. 20, p. 289) crystallises from alcohol in plates, from ether in needles. M. p. 154°.

Examination of the Excrement of the Rabbit.

The material was obtained in the summer from wild rabbits. Treated as before, from 3 kilogrammes of the dried fæces, 6·2 grammes, or 0·21 per cent., of pure hippocoprosterol were obtained. On analysis it gave the following results :—

0·1656 gave 0·4974 CO_2 and 0·2135 H_2O.

	Found.	Calculated for $C_{27}H_{24}O$.
C	81·91	82·14
H	14·33	13·80

The oils left after complete separation of the solid matter weighed 23·26 grammes, or 0·77 per cent.

Mr. G. W. Ellis, at our suggestion, attempted to ascertain the composition of these residues by a fractional distillation *in vacuo,* but the process proved tedious and difficult and led to no very definite results. At first the liquid simply frothed over, but on returning the distillate and repeating several times the frothing became less marked, and under a pressure of less than 1 mm. it distilled over between 98° and the temperature at which the glass softened without the slightest charring. After elaborate and repeated fractionation, four main fractions were obtained, boiling around the following temperatures:—(1) 98°, (2) 164° to 168°, (3) 215° to 220°, (4) 260° to 265°. In the flask there remained a transparent, yellow, brittle, resinous substance which was not decomposed at the softening point of glass.

Fraction 1 consisted of about 2 c.c. of a pale yellow, fairly mobile oil, with a smell recalling that of pine oil.* On combustion it was found to contain 82·13 per cent. carbon and 11·96 per cent. hydrogen.

Fraction 2 consisted of about 3 c.c. which, on long standing, deposited a trace of crystalline matter. It had a very faint turpentine odour and reduced ammoniacal silver solution in the cold, markedly on heating.

Fraction 3 consisted of about 5 c.c. of a very thick oil, smelling faintly of hay, and only just mobile at the ordinary temperature.

Fraction 4 was the largest and consisted of a pale yellow, sticky, solid (at the ordinary temperature), which showed no signs of crystallisation after many months' standing. On combustion it gave the following figures, which agree closely with those required for cholesterol :—

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* The rabbits from which these fæces were obtained lived on the border of a pine wood.

I. 0·2449 gave 0·7562 CO_2 and 0·2547 H_2O.
II. 0·2694 „ 0·8343 CO_2 „ 0·2908 H_2O.

	Found.		Calculated for
	I.	II.	$C_{27}H_{44}O$.
C	84·21	84·05	84·37
H	11·56	11·99	11·46

All attempts to prepare a crystalline acetate or benzoate of this substance failed.

In the course of the investigation we noticed that samples of fæces collected during the winter season, when the animals were not fed entirely on grass, gave a lower yield of hippocoprosterol. Furthermore, an examination of the fæces of domestic rabbits, fed on cabbage, made for us by Mr. G. D. Knox, showed that with this food a different product was obtained, an account of which we reserve for a future communication. We therefore were led to suspect that the hippocoprosterol might be a constituent of the grass on which the animals fed.

Examination of Grass.

3·2 kilogrammes of the blades of grass obtained from the cuttings of a well-kept cricket pitch, fairly free from clover and other plants, were dried in thin layers in the air and extracted as before. The extract was dissolved in alcohol and decolorised with charcoal. The solution was now pale yellow and deposited a gelatinous solid mass which was readily worked up by methods previously described and obtained pure. The yield was 8 grammes, or 0·27 per cent., of the dry material. This body proved identical with hippocoprosterol, an important point which was confirmed by the following experiments:—

(*a*) six grammes of the body were crystallised from benzene into three fractions, *each* of which gave a melting and solidifying point (79° and 77° respectively), the same as that of hippocoprosterol.

(*b*) Equal weights of the body from grass were mixed separately with equal weights of that from the horse, cow, sheep, and rabbit. The melting points remained unchanged. Finally, a sample from each of the five sources was mixed and the same result obtained.

(*c*) The acetate and benzoate were made as before and proved identical with those from hippocoprosterol. They were saponified and yielded hippocoprosterol, melting at 79°.

(*d*) On analysis, the following figures were obtained:—

I. 0·1924 gave 0·5758 CO_2 and 0·2464 H_2O.
II. 0·1742 „ 0·5234 CO_2 „ 0·2230 H_2O.

	Found I.	Found II.	Calculated for $C_{27}H_{54}O$.
C	81·62	81·94	82·14
H	14·23	14·22	13·80

The mother liquors from this product yielded a considerable quantity of reddish oily matter, similar to that found in the fæces.

It would, therefore, appear that hippocoprosterol is not an animal product, but is a constituent of the grass food which is passed unchanged. In order to confirm this conclusion, and also to ascertain whether any cholesterol or derivative of it which we might have missed in our previous experiments was excreted by the animal, we made a series of experiments in which a domestic rabbit was fed on grass which had been thoroughly extracted with ether.

Experiment 1.—A rabbit, weighing 2·1 kilogrammes, was fed with 315 grammes of extracted grass, slightly moistened with water, during 14 days. A very small quantity of bran was given in addition. The animal took the grass readily, and at the end of the experiment had only lost 0·1 kilogramme in weight, and appeared to be in good health. The weight of dry fæces obtained was 128 grammes. This was extracted in the usual way, and the extract was found to have a somewhat fœtid odour not noticed with ordinary dung. One gramme of unsaponifiable matter was obtained, the greater portion of which was soluble in alcohol. On standing, a very small quantity of red crystalline matter was deposited, but the bulk of the substance eventually separated as a non-crystalline red oil. No trace of hippocoprosterol was discovered, and the small quantity of crystalline matter referred to after purification was obtained from dilute alcohol in the form of glancing white leaf-like crystals and from ethyl acetate as needles, which melted rather indefinitely at about 129°. Under the microscope the crystals from alcohol showed the form of hexagonal plates, recalling those of phytosterol, but the quantity was too small for further investigation.*

Experiment 2.—A rabbit, weighing 2·1 kilogrammes, was fed during 18 days on extracted grass with a little bran. The quantity consumed weighed before extraction 2·8 kilogrammes, and was of a somewhat coarser description than that previously used; 982 grammes of dried fæces were obtained, and were treated as before. The residue obtained weighed 5·64 grammes. From this we isolated 0·25 gramme of hippocoprosterol, and the mother liquors on evaporation deposited an oil, which on standing showed under the microscope traces of crystalline matter in the form of six-sided plates and clusters of sword blades, but in too small quantity for further examination and identification.*

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* This substance was derived from the bran given to the animals, as on extraction of a sample of bran we obtained a body, crystallising in the same forms, which melted at 137°·5 C., and appeared to be identical with Burian's sitosterol.

General Conclusions.

1. Hippocoprosterol isolated from the fæces of the horse by Bondzynski and others is not a product of animal metabolism, but is a constituent of the grass taken as food, and is passed unchanged by all herbivorous animals when fed on grass. The name is, therefore, misleading, and we propose to rename the substance chortosterol (χόρτος, grass).

2. Chortosterol is an alcohol having the formula $C_{27}H_{54}O$ or $C_{27}H_{56}O$. It is not possible at present to decide definitely between these, though our analyses in every case agree better with the former.

3. If we consider the numerous vegetable cholesterols which have the properties of unsaturated monatomic alcohols and the formula $C_{27}H_{44}O + H_2O$ as isomeric substances constituting the phytosterol group, chortosterol cannot be regarded as a simple reduction product of any one of them in the same way that coprosterol is supposed to be related to cholesterol. It is possible that the substance may be derived from some member of this group, or *vice versâ*, by some rearrangement of the ring structure during the development of the plant. We are at present engaged in some experiments on this point. This is, perhaps, supported by the fact that chortosterol, unlike other members of the cholesterol or phytosterol groups, gives none of the usual colour reactions, for Windaus† has shown that when the unsaturated open side chain of the cholesterol and phytosterol molecules is condensed to a ring, the products obtained show the colours feebly‡ or not at all.

4. In all the experiments we have made we have never found any cholesterol in the fæces of the herbivora. If the view of Flint and other observers that cholesterol is an excrementitious product got rid of in the fæces through the agency of the bile, we should certainly have expected

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* This substance was derived from the bran given to the animals, as on extraction of a sample of bran we obtained a body, crystallising in the same forms, which melted at 137°·5 C., and appeared to be identical with Burian's sitosterol.

† 'Ber.,' vol. 40, pp. 2637 and 3681 (1907).

‡ *Cf.* Diels and Abderhalden, 'Ber.,' vol. 39, p. 884 (1906).

in the very large quantities of material examined to have obtained considerable quantities of cholesterol as such, or in a modified form as in the human subject. In the cow, for instance, every 100 c.c. of bile contains approximately 0·07 gramme of cholesterol, and supposing in this animal only 2½ litres are poured into the intestine per day, this would mean a daily excretion of nearly 2 grammes, which we could not possibly have failed to discover. It follows, therefore, that cholesterol of the bile must either have been reabsorbed with the bile salts in the gut, or else destroyed. We are at present carrying out experiments on this subject with herbivora and other animals, an account of some of which we hope shortly to have the honour of laying before the Society.

This work has been carried out with the help of a grant which was made to us by the Government Grant Committee of the Royal Society, for which we take this opportunity of expressing our thanks.

The Origin and Destiny of Cholesterol in the Animal Organism. Part II.—*The Excretion of Cholesterol by the Dog.*

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(From the Physiological Laboratory, University of London.)

In a former paper* we showed that the so-called hippocoprosterol, which was considered by its discoverers† to be a reduced cholesterol, and the form in which the cholesterol of the bile of the horse is excreted in the fæces, is contained in the solid excreta of all grass-fed herbivorous animals, and that it is not a product of the animal metabolism, but a constituent of the grass taken as food and passed unchanged. We also showed that cholesterol is not found in the fæces of these animals. It therefore seemed to us desirable to extend the investigation to carnivora, and to ascertain, if possible, whether the cholesterol often found in their excreta is derived from the organism or from the food, and whether, under any circumstances, it is found in modified form as in the human subject.

Of the various animals the dog appeared to be the most suitable for initial experiments, as it is easily trained and will thrive not only on meat but on vegetable diets. The animal selected for experiment was an Irish terrier bitch, between four and five years of age, and weighing 11·8 kilogrammes. It was quiet and used to a sedentary life. Preliminary experiments, using a mixed diet, showed that the quantity of cholesterol excreted per day was exceedingly small, much too small for even approximate estimation. We therefore decided to keep the animal under observation for the greater part of a year, and to feed it on particular diets for many consecutive days, varying from 14 to 30, according to circumstances. We did not think it necessary at this stage of the enquiry to make any determinations on the nitrogen metabolism during the experiments, but we took great care to keep the animal in good health during the whole time, by regular exercise, periods of rest, etc. During a part of the time the dog was kept at Messrs. Ridler and Hobday's establishment, and we take this opportunity of expressing our

* 'Roy. Soc. Proc.,' this volume, p. 212.

† Bondzynski and Humnicki, 'Zeit. Physiol. Chemie,' vol. 22, p. 396.

thanks to Professor Hobday for the care and attention bestowed on the animal while there.

The fæces collected during each diet period were dried in the water oven, roughly powdered, and extracted thoroughly in a Soxhlet's apparatus with ether. The extract in dilute ethereal solution was then saponified by sodium ethylate, and the precipitated soaps filtered off and well washed with ether. The ethereal filtrate so obtained was repeatedly shaken with water containing soda, to get rid of alcohol, some colouring matter, and excess of soap. It was then dried and the ether distilled off. The unsaponifiable matter differed widely with different diets in quantity and in character, being in some cases a viscid oil, and in others solid.

We hoped to be able to separate the cholesterol from this unsaponifiable matter by conversion into the dibromide, and taking advantage of the sparing solubility of the latter substance in petrol, but preliminary experiments showed that with the small quantities of cholesterol obtained the method was of no value from a quantitative point of view. We found it better to crystallise fractionally from absolute or from 85 to 90-per-cent. alcohol. The cholesterol was identified by microscopical examination, by the melting point after recrystallisation, and by conversion into the benzoate and the acetate. It was found better to convert the smaller crops of crystalline matter at once into benzoate, as this substance is very sparingly soluble in absolute alcohol.

The acetate of cholesterol is readily prepared by boiling cholesterol with acetic anhydride and sodium acetate for a few minutes, pouring into water, and recrystallising from alcohol. The benzoate can be prepared by heating to 140° for half an hour or more with benzoyl chloride, treating the product with hot alcohol, and recrystallising from alcohol. We find, however, that it is a much better method in the case of cholesterol to dissolve this substance in pyridine in the proportion of about 1 gramme to 10 to 20 c.c., add a solution of a little more than the theoretical quantity of benzoyl chloride in pyridine solution, and allow to stand for several hours. The liquid is then poured into water, when the benzoate of cholesterol is precipitated, and may be recrystallised from absolute alcohol. The yield by this method is quantitative. The acetate of cholesterol melts at 114° C., and on cooling shows colour changes if pure. The benzoate melts at 145°·5 C. to a turbid liquid, which suddenly becomes clear at 178° to 180°, and, in cooling, a brilliant display of opalescent colours is exhibited, among which a brilliant blue, appearing at about the temperature of the higher melting point, followed by a violet blue just before complete solidification, are most prominent. These colour changes are well marked and very characteristic of cholesterol.

The non-crystalline oily matters obtained from all the mother liquors in the above-mentioned fractional crystallisation processes were treated in pyridine solution with benzoyl chloride, in order to separate as benzoate any cholesterol that might have been retained in solution in the oils. In a few experiments, small amounts of cholesterol benzoate were obtained in this way from these oils, but in most cases none was found. The following is a brief account of the results of our experiments, using seven different diets:—

(a) *Oatmeal Porridge and Milk.*—The oatmeal was well boiled with water containing a little salt, and milk added. During 21 days the dog consumed 3865 grammes of coarse oatmeal—or 184 grammes per day, and 2340 c.c. of cow's milk, *i.e.*, 128 grammes per day. During this period 1539 grammes of natural undried fæces were obtained.

On saponification in the manner described, a very large quantity of slimy soap was obtained, which was difficult to filter and wash with ether, and consequently had to be dried and re-extracted in the Soxhlet with ether. The unsaponifiable matter was obtained in the form of a red viscid oil, having a slight odour of peppermint. This oil was for the most part soluble in hot acetone, leaving a small amount of red slimy matter. On standing, the acetone deposited highly coloured and somewhat sticky crystalline matter. This was recrystallised from alcohol, when 0·95 gramme of brown crystalline matter was obtained. The alcoholic mother liquors, on standing, deposited some oily substance, and finally, on evaporating to small bulk, 0·35 gramme of sandy amorphous solid, which would not crystallise. The 0·95 gramme of coloured crystalline matter was recrystallised several times from 85-per-cent. alcohol, and was eventually obtained in three fractions, white in colour, and melting respectively at 134°, 137° to 139°, and 132° to 133°. The total weight was 0·5 gramme. A microscopic examination showed that these crystals consisted entirely of cholesterol. This was confirmed by conversion into the benzoate, which in each case melted correctly, and showed the colour changes in a well-marked manner. All the mother liquors were evaporated to dryness, and gave a stiff red oil. This dissolved for the most part in cold petrol, leaving 0·1 gramme of a red sandy solid melting at 120° to 130°.

On evaporating off the petrol, a viscid oil was obtained, which, on long standing, set to a crystalline waxy mass, weighing 1·25 grammes. This wax was dissolved in ethyl acetate, and, on standing, long white needle-shaped crystals separated, mixed with red colouring matter. The weight of crude crystals was 0·28 gramme. They were rather difficult to purify, but were eventually obtained in the form of glistening pearly leaflets easily soluble in ethyl acetate, but insoluble, or difficultly soluble, in petrol. This substance began to soften and sinter at 120° and melted sharply at 134° C. This was not cholesterol and was probably a phytosterol contained in the meal. The mother liquors yielded no further crops of crystalline matter, and dried to a viscid oil, which, on standing, showed no sign of crystallising. The yield of cholesterol was therefore about half a gramme or 0·023 gramme per day.

(b) *Cooked Beef and Mutton.*—After the conclusion of the last experiment the dog was fed for a week or more on mixed diet, and given regular exercise, and appeared to be in good condition. It was then fed for 20 days on cooked beef and mutton with an occasional bone. The meat was fairly lean, but the fat was not specially taken out. The total weight consumed during the 20 days was 7470 grammes, or 373·5 grammes per day; 413 grammes of undried fæces were passed during this period, and, after treatment, yielded 2·1 grammes of unsaponifiable matter in the form of an oily solid. This was dissolved in alcohol, partially decolorised with charcoal, and fractionally crystallised; 0·615 gramme of crude cholesterol was obtained. It was moderately pure, and, after recrystallisation from 85-per-cent. alcohol, melted at 135° to 138°. It gave a satisfactory yield of benzoate which melted rather high, 147°·5, to a turbid liquid which cleared at 160°, and, on cooling, showed the colour changes in a well-marked manner. The mother liquors deposited about 1 gramme of a brown sticky solid which would not crystallise. It was treated in pyridine solution with benzoyl chloride, but gave no trace of cholesterol benzoate.

The yield of cholesterol was therefore about 0·615 gramme, or 0·037 gramme per day.

(c) *Sheep's Brain.*—After the last experiment the dog was kept on mixed diet and allowed regular exercise for one month, when it was in excellent health. It was now given a diet rich in fatty matter and cholesterol—raw sheep's brain—which was continued for 14 days. During this period the dog consumed 28 sets of brains, weighing in all 2129 grammes, and appeared to relish the food. It was also allowed 170 grammes of bone; 316 grammes of undried fæces were passed. The unsaponifiable matter was obtained as a buttery mass, weighing about 30 grammes. This, however, was not dry. On crystallisation from alcohol, three fractions were obtained, weighing respectively 9·8, 5·3, and 1·2 grammes. These were only slightly coloured and under the microscope appeared to consist entirely of long pointed needles: no typical cholesterol crystals could be seen. Each fraction, after recrystallising once from alcohol, appeared pure, and melted at about 96° C. On a careful fractional crystallisation no trace of higher melting bodies could be obtained. This coprosterol was saturated to bromine. The acetate melted at 88° to 89°, and the benzoate at 122° to 123° to clear liquids showing no colour effects on cooling. Three crops of crystals, examined with the polariscope in chloroform solution, gave the following results :—

$$(1)\ [\alpha]_D = +23^\circ{\cdot}07,\quad (2)\ [\alpha]_D = +23^\circ{\cdot}7,\quad (3)\ [\alpha]_D = +22^\circ.$$

It thus appears identical with the coprosterol of human fæces described by Bondzynski and Humnicki.* To confirm this point, the whole of the material was converted to the acetate, which was repeatedly crystallised from alcohol and acetone. The highly pure ester was saponified with sodium ethylate in ethereal solution, and the product twice recrystallised from alcohol. A sample of human coprosterol was similarly worked up and purified, and in the following table we give a comparison of their properties.

	Coprosterol, dog.	Coprosterol, human.
Crystallises from alcohol in	Long flexible needles	Long flexible needles.
Melting point	Falls together at 98° ; melts, 99°—100°	Falls together at 98° ; melts, 99°—100°.
Rotation $[\alpha]_D$ in chloroform	+ 23° ·7	+ 23° ·5.
Acetate............................	Needles from alcohol ; m. p., 88°—89°	Needles ; m. p., 88°—89°.
Benzoate (prepared by the pyridine method)	Leaves, difficultly soluble in alcohol ; m. p., 122°—123°	Leaves, difficultly soluble in alcohol ; m. p., 121°—122°.

The mother liquors, on standing, set to a brown oily mass. This was put on a porous tile and the oily part drained away. The dry solid matter was heated to 140° with benzoyl chloride, and then treated with hot alcohol. It dissolved readily with the exception of a small quantity which was only got into solution with difficulty. On cooling, about 0·2 gramme of white crystalline matter separated. This was recrystallised from alcohol and melted at 141° to a turbid liquid, which on cooling showed the brilliant colours of cholesterol benzoate. The mother liquors, on spontaneous evaporation, yielded a small quantity of brown crystalline matter. This was very easily soluble in absolute and dilute alcohol, but was not further investigated.

The yield of coprosterol was thus 16 to 17 grammes, or 1·14 to 1·2 per day.

(d) *Cooked Horseflesh.*—After the last experiment the dog was allowed to rest for a month, and was then fed for a month on porridge made from coarse oatmeal, and water. The product was unfortunately lost. The animal was then given another month's rest with an ordinary mixed diet. At the end of this period it was fed for 17 days on cooked horseflesh with scarcely any fat. In the middle of this experiment there was an interval of four days, during which the dog had a diet of dog biscuit, but the fæces were not collected during this interval. During the period it consumed 6758 grammes of meat, or 397 grammes per day. With a view to sweeping out the gut at the end, it was fed for two days on bread and bovril; 388 grammes of fæces were collected, which on drying at 80° weighed 155 grammes; 3·47 grammes of unsaponifiable matter in the form of a red oil were obtained; this was very liquid at 100°, and had a soapy smell. With the exception of a little tarry matter this was soluble in alcohol, and, on evaporation, two crops of crude cholesterol were obtained, weighing respectively 0·67 and 0·074 gramme.

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* 'Zeit. Physiol. Chemie,' vol. 22, p. 396.

This was identified by conversion into the benzoate, and the yields showed that it consisted practically entirely of cholesterol.

The mother liquors, on evaporation, yielded oil, which, on treatment with benzoyl chloride in pyridine solution, yielded 0·54 gramme of brown crystalline matter which, after recrystallisation from ethyl acetate, melted to a turbid liquid at 142° to 145°. This cleared at about 180°, and on cooling showed the characteristic colour changes of cholesterol benzoate. The total yield of cholesterol was, therefore, approximately 1 gramme, or 0·06 gramme per day.

(e) *White of Egg, Bread, and Cream.*—Immediately after the conclusion of the last experiment the animal was put on the above diet. The daily ration was prepared by mixing about 150 grammes of bread with the whites of three eggs and half a teaspoonful of cream. This was fried and moistened with a warm dilute solution of Liebig's extract of beef. This diet was continued for 14 days, during which the animal consumed 2092 grammes of bread and the whites of 42 eggs. This proved a very nourishing diet, and at the end of the experiment the dog was in excellent condition; 130 grammes of fæces were passed, and weighed, after drying at 100°, 35 grammes. On saponification, the soap was white in colour and not large in amount. The ethereal solution of the unsaponifiable matter was pale yellow, and on evaporation gave 0·59 gramme of a sticky yellow solid. This, with the exception of a small amount of tar, was soluble in 90-per-cent. alcohol, and on cooling the solution set to a mass of crystals. A small portion was examined under the microscope, and appeared to consist of small rosettes of needles, but no cholesterol crystals were at first observed. On dissolving again on the slide and recrystallising, a small patch of typical cholesterol crystals was observed, but the bulk consisted of the rosettes referred to. The total weight of crystalline matter was 0·3 gramme. This was decolorised by animal charcoal, and crystallised from the least quantity of 85-per-cent. alcohol.

The first and main crop of crystals, after drying at 100° C., melted at 137° to 140°. The acetate, after several recrystallisations, melted at 120° to 122°, and no colour changes were noticed. The benzoate began to soften at 138°, and melted to a *clear* liquid at 142° to 143°. On cooling, *no colour changes could be observed.* The substance was probably sitosterol, derived from the bread.

The second and smaller crop from the mother liquors of the above was decolorised and recrystallised from alcohol. This was not pure, and the quantity was too small for further purification. It began to melt at 120°, but the process was not complete at 135°. It was converted into the acetate, which after recrystallisation melted sharply at 113° to 114° C. This was probably cholesterol acetate, though no colour changes were noticed with certainty. The soapy matter left on evaporating the mother liquors was treated with benzoyl chloride in pyridine solution, but no crystalline matter could be obtained. In this experiment, therefore, only traces of cholesterol were obtained.

(f) *Oatmeal Porridge.*—As the dog at the conclusion of experiment (e) was in excellent health, it was at once put on a diet of porridge made from coarse oatmeal and water with a little salt. This diet was continued for 31 days, during which the dog consumed 2870 grammes of oatmeal; 212 grammes of dried fæces were obtained. The ethereal extract, measuring about 1·5 to 2 litres, on saponification set to an almost solid mass of soap. This, however, filtered easily, and was readily washed. The unsaponifiable matter was a reddish brown oil, weighing 2·7 grammes. With the exception of 0·27 gramme of tarry matter this was soluble in alcohol. On standing, the solution deposited a slimy brown mud. This was taken up in ethyl acetate, and, on standing, the solution deposited several crops of white needle-shaped crystals, the total weight being 0·255 gramme. The ethyl acetate mother liquors, on spontaneous evaporation, yielded an oil.

The needle-shaped crystals were readily soluble in petrol and in alcohol, but did not crystallise readily from these solvents. After several recrystallisations from ethyl acetate the substance was obtained in the form of long, flat, slender needles, which melted sharply at 133° to 134° C. The acetate began to soften at 70°, and melted to a clear liquid at 98° C., but we had not enough for a satisfactory purification. It was evidently the same as the phytosterol obtained in experiment (a). The alcoholic mother liquors from the above-mentioned muddy solid, on spontaneous evaporation, gave an oil through which crystalline matter was disseminated. This was recrystallised from 85-per-cent. alcohol when 0·172 gramme of whitish crystals was deposited. Under the microscope these were found to consist of long hexagonal plates, and no typical cholesterol crystals were observed. After recrystallising from absolute alcohol until quite white, the substance melted at 124° to 125°. The acetate crystallised in microscopic plates, which melted at 118° to 122°. These properties agree with those of the para-sitosterol of Burian.

All the mother liquors, from which the above-mentioned crystalline substances were obtained on spontaneous evaporation yielded viscid oils, which really constituted the bulk of the unsaponifiable matter. In order to ascertain whether any cholesterol or other crystalline substances were held in solution in these oils, they were dissolved in pyridine and treated with benzoyl chloride. After standing, the solutions were poured into water and the slimy deposits dissolved in hot absolute alcohol and left to crystallise. One oil yielded 0·094 gramme and another 0·04 gramme of crystalline matter, difficultly soluble in absolute alcohol. On purification, this was obtained in the form of glistening plates which melted at 145° to a turbid liquid. This cleared at about 175° and on cooling showed the colour changes characteristic of cholesterol benzoate. The mother liquors, on spontaneous evaporation, dried to a viscid oil, which, on long standing, showed no sign of crystallising. Only about 0·1 gramme of cholesterol was therefore obtained in the month, or 0·003 gramme per day.

(g) *Rice, Gelatine, and Butter.*—After experiment (f) the dog was fed for four or five days on dog biscuit and porridge and given regular exercise, and as it appeared to be in good health, the final experiment was proceeded with. Each day's ration consisted of 114 grammes of rice, which was well boiled and mixed with 114 grammes of a 10-per-cent solution of gelatine and 28 grammes of butter. The mass was flavoured with a dilute solution of Liebig's extract of meat. During 18 days the dog consumed 2052 grammes of rice, about 205 grammes of gelatine, and 504 grammes of butter. The weight of dried fæces was 115 grammes.

1·316 grammes of unsaponifiable matter was obtained as a vaseline-like oil. This, with the exception of 0·06 gramme of tarry substance, dissolved in 50 c.c. of hot 90-per-cent. alcohol. Three crops of impure crystalline matter were obtained, (I) 0·37 gramme; (II) 0·16 gramme; and (III) 0·14 gramme. The mother liquors dried to an oil, which, on long standing, showed no sign of crystallising. Crop I, after decolorising and recrystallising from 90-per-cent. alcohol, was obtained in the form of white crystals not unlike cholesterol in appearance. On heating, these began to soften at 120° and melted at about 129°. A microscopic examination showed that the substance was a mixture. We tried to separate the substances by means of the benzoates. The crude benzoate obtained by the pyridine method crystallised from alcohol, apparently in two forms, one light and floating in the liquid and the other granular and adhering to the sides of the beaker. These were separated as far as possible and recrystallised separately. The light form, after recrystallisation from alcohol, in which it was sparingly soluble in the cold, melted at 139° to a pale brownish liquid which was slightly turbid, but not so markedly as is usually the case with cholesterol benzoate and did not become any clearer at 170° to 180°. On cooling, no colour changes were observed at the higher temperature, but at the point of solidification a transient lilac blue appeared, but was not well marked. This substance might have been impure cholesterol benzoate, but we could not be certain.

The granular portion, which was the greater quantity, after recrystallisation shrank together at 138° C. and melted sharply at 139° C. to a *perfectly clear liquid.* It showed no colour changes and was not cholesterol benzoate.

Crop II was decolorised by charcoal and recrystallised from alcohol. It began to soften at 120° and melted at 135°. It was mostly cholesterol, for the benzoate made by the pyridine method melted at 140° to 143° to a turbid liquid which went clear at 178° and on cooling showed the colour changes in a well-marked manner.

Crop III was very impure and greasy. It was therefore treated at once with pyridine and benzoyl chloride. A minute amount of crystalline matter was obtained which melted at 145° to a turbid liquid and appeared to clear at 178°. It was, however, yellow in colour, and we could not observe the colour changes. The oils from the mother liquors were benzoylated, but no crystalline matter was obtained. In this experiment, therefore, 0·667 gramme of impure crystalline matter was obtained. This contained some cholesterol, but we could not determine the amount. We do not think that more than one-third of it was cholesterol, certainly not one-half. The rest evidently consisted of phytosterols from the rice.

Discussion of Results.

Different observers have given very variable figures for the daily quantity of bile secreted by the dog, as the following table shows :—

Observer.	Quantity of bile in grammes secreted per kilogramme-weight in 24 hours.
Friedlander and Barisch	19·9
Bidder and Schmidt	24·5
Heidenhain	32·7
Dastre	10·0

If we take the mean of the above results as approximately correct, the daily secretion of bile by our dog would be 291 grammes.

For the content of cholesterol in 100 parts by weight of dog's bile, Hoppe-Seyler gives the following figures:—

(*a*) Bile from bladder.		(*b*) Bile from fistula.	
I.	II.	I.	II.
0·449	0·133	0·074	0·049

We do not know how far the bile poured into the gut would resemble either bladder bile or fistula bile, but, calculating on the basis of the lowest and highest values given, our dog should have received into its intestine, along with the bile, between 0·14 and 1·31 grammes of cholesterol per day. If it be the case that cholesterol is a true excretion product got rid of in the fæces through the agency of the bile, this quantity should have been found in the excreta along with that contained in the food consumed, whereas, as shown in the following table, in which we compare the amounts of cholesterol actually found in our experiments with those that should have been found on the assumption that the cholesterol of the bile is all excreted in the fæces, the amounts found in most cases were less than one-fifth of the lowest of the above values.

It is clear from the above experiments that the whole of the cholesterol of the bile is not excreted in the fæces. It must, therefore, have been either totally destroyed, which, considering the great stability of cholesterol, is highly unlikely, or reabsorbed in the gut along with the bile salts, which is the more reasonable explanation. It might be contended that the cholesterol had been changed in the intestine into the unsaponifiable oil found along with the cholesterol, but this is probably not the case, as the oils obtained were very variable, both in quantity and appearance, and further, the weights of such oils obtained were usually quite insufficient to account for the cholesterol.

How far the quantities of cholesterol found can be accounted for as cholesterol contained in the food and passed unchanged is more difficult to answer, as we have little exact knowledge of the cholesterin content of the various food stuffs. In our opinion, however, it can be largely accounted for in this way.

Considering the two experiments with cooked meat, in which the cholesterol recovered for 20 days was only 0·6 and 1·0 respectively, these amounts might well have been due to the food taken. As is well known, animal flesh contains cholesterol, though quantitative estimations have, as far as we are aware, not been made. Such estimations would be tedious and

Diet.	Total weight of solid food in grammes.	Number of days.	Total weight of fæces undried in grammes.	Total weight of fæces dried at 100° C. in grammes.	Total weight of unsaponifiable matter in grammes.	Total weight of cholesterol found in grammes.	Amount of cholesterol that should have been excreted from the bile on above assumption.	Amount of cholesterol passed per day in grammes.	Output of cholesterol per day that should have been produced from bile only.
A. Oatmeal and milk	4147	21	1539	—	—	0·5	2·94—27·5	0·023	0·14—1·31
B. Cooked beef and mutton	7470	20	413	—	2·1	0·615	2·8 —26·2	0·037	0·14—1·31
C. Raw sheep's brain	2129	14	316	—	30·0 (not quite dry)	16—17*	1·9 —18·3	1·2*	0·14—1·31
D. Horseflesh......	6758	17	388	155	3·47	1·0	2·38—22·27	0·059	0·14—1·31
E. Bread, white of egg, and cream	2092 and 40 egg whites	14	130	35	0·59	Trace	1·9 —18·3	Small traces	0·14—1·31
F. Oatmeal.........	2870	31	—	212	2·7	0·1	4·34—40·6	0·003	0·14—1·31
G. Rice, gelatine, and butter	2052 rice 205 gelatine 504 butter	18	—	115	1·3	Not more than 0·3	2·5 —23·6	0·017	0·14—1·31

* Coprosterol, not cholesterol.

difficult to perform, as they would involve the artificial digestion of large quantities of material, for it has been shown that it is impossible to extract the whole of the fatty matter from meat without such digestion. Furthermore, the quantities would probably have been variable. In the two experiments quoted, however, the cholesterol found would only have meant, in the case of beef and mutton, a content of 0·008 per cent., and in the case of horseflesh of 0·015 per cent.

In experiment (f), with an oatmeal diet, we found only 0·1 gramme of cholesterol. Whether oatmeal contains any traces of cholesterol we cannot say, but on extracting three days' diet we were unable to recognise any in the unsaponifiable residue. Taking this 0·1 gramme as a basis for calculation in experiment (a) (oatmeal and milk), the yield of cholesterol attributable to oatmeal would have been 0·135 gramme. Milk fat, according to Schmidt and Mulheim, A. Bömer, and A. Kersten, contains 0·5 per cent. of crude cholesterol, which Menozzi has shown to be identical with that of bile. If we take our milk as containing between 3 and 4 per cent. of fat, the amount of cholesterol from this source should have been for the quantities taken in experiment (a) between 0·35 and 0·468 gramme. The total cholesterol to be expected would therefore be between 0·485 and 0·6 gramme. We actually found about 0·5 gramme.

In experiments (e) and (g) the traces of cholesterol found could be accounted for as due to the food.

Experiment (c), on a diet of sheep's brain, is of special interest, not only because of the quantities obtained, but because the cholesterol was entirely in the form of coprosterol. The brain substance is well known to be rich in cholesterol. The human brain contains about 2 per cent. Whether the content of cholesterol in the brain of other animals is as high as this we do not know. On the assumption that it is the same as in the human brain we should have expected in the fæces of a period of 14 days between 25 and 30 grammes of cholesterol. We actually found 16 to 17 gr. of coprosterol. There can be no doubt that this was due to the diet. In man, according to Bondzynski and to Müller (and in agreement also with our own experience), the change of cholesterol into coprosterol in the gut by bacteria, is the normal process, and if the gut is cleared of the particular bacteria by prolonged milk diet, cholesterol is excreted as such.

In the dog, fed on either cooked vegetable or meat diet, cholesterol is normally excreted as such. In the case of raw brain we obtained coprosterol only. The cause of this we must reserve for future investigation. It may have had something to do with the fact that the food was uncooked, or that it was unusually rich in fat. Unfortunately, we were unable to ascertain whether the state of the gut favourable to the formation of coprosterol persisted when the next diet (oatmeal) was tried, as, owing to an accident, the whole of the ethereal extract was lost. In the subsequent experiment with horseflesh, the gut had recovered its normal condition.

Whether any of the cholesterol of the food is actually absorbed along with that of the bile in the intestine, these experiments do not show, but others are in progress, which we hope will throw light on this point, and this we hope to make the subject of a communication in the near future.

The expenses in connection with this work were defrayed by means of a grant made by the Government Grant Committee of the Royal Society, for which we take this opportunity of expressing our thanks.

Bacteria as Agents in the Oxidation of Amorphous Carbon.

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The problem which presented itself to my mind in commencing the following investigation was primarily one connected with agriculture. When considering the application of such insoluble substances as charcoal, cinders, soot, etc., to the land, an explanation was sought of their ultimate fate in the soil. What becomes of the carbon ? Is it oxidised into CO_2, and if so by what agency ?

Little is known at present as to the means whereby amorphous carbon is rendered available for plant life, except through its union with oxygen in the process of combustion, and further investigation upon this point offered an important field of enquiry. It is well established that carbon readily absorbs oxygen, and, in the case of coal, that carbonic acid is given off, but the cause of the latter phenomenon is still obscure, and in the theories advanced to account for it no consideration is ever given to the possible action of micro-organisms.

My investigations have shown that under the action of certain bacteria a slow oxidation of amorphous carbon takes place, CO_2 is slowly evolved, and the carbon can thus be at once utilised for the nutrition of green plants.

This led to a wider consideration with regard to the action of bacteria upon certain carbon compounds such as coal and peat, and opened up the question as to whether it was possible that the vast supplies of carbon locked up in these formations could be utilised for plant life without the intervention of direct combustion. That coal undergoes considerable wastage when exposed to the air is a fact very generally known, and in the case of large storages of coal the depreciation may reach a very high percentage. The conditions often preclude this loss being entirely attributed to weathering, and the question arises: are there then any other agents, such as bacteria, concerned in the process of disintegration?

In experiments dealing with substances of the nature of coal, peat, and charcoal, quite peculiar care had to be taken for the purpose of sterilisation, which proved to be a very difficult matter; while the chemical changes which take place during heating these substances to such a degree as was necessary, and the probable conservation of gases, were all points requiring due consideration for the elimination of possible sources of error. Further, the highly sensitive character of the electrical apparatus employed rendered special precautions necessary to guard against extraneous influences. It will therefore be needful to give in detail, though as shortly as possible, the methods employed in conducting my research.

The problem was attacked by three distinct methods, briefly stated as follows:—

1. By passing a stream of air, freed from all trace of CO_2, over the material subject to investigation and determining the presence of CO_2 by titration with standard oxalic and hydrochloric acids.

2. By determining the rise of temperature due to oxidation by means of a thermopile and galvanometer.

3. By detecting, in the case of charcoal, the presence of calcium carbonate in the flasks inoculated with the bacteria.

Charcoal.

Pieces of ordinary wood-charcoal were pounded and passed through a double sieve, the first having a mesh of 1/10 inch, the second of 1/20 inch, so that in this way small fragments of fairly uniform size were retained between the two. A quantity of this charcoal was next heated by means of a metallurgical furnace to a white heat (about 1200°), in a crucible protected from any access of atmospheric oxygen. Heating to this point was a necessary treatment, as commercial charcoal is seldom sufficiently charred to drive o all volatile compounds. The entangled oxygen would thus, in the presence of an excess of carbon, be mainly converted into CO, and the calcium salts naturally contained in the woody tissue into the oxide. This process was also important as an efficient means of sterilisation, and for the destruction of any organic matter in the shape of dust with which the material might be contaminated.

Determination of CO_2 by Titration.

The *apparatus* employed was in main outline that used by Sachs in his classical experiment on Respiration, with modifications to suit the special difficulties in this case. After some preliminary trials the form of apparatus finally adopted was as follows :—

Air drawn by means of an aspirator was passed first through a Reiset absorption apparatus containing 100 c.c. of a strong solution of caustic soda, next through a similar apparatus containing an equal volume of baryta water, then through the flask containing the material under investigation (known henceforth as the research-flask), and finally through a Reiset containing 100 c.c. of baryta water.

To avoid corks and the consequent difficulty of ensuring their perfect sterilisation, specially constructed research-flasks (Cloëz flask) were employed, in which the delivery tube, reaching nearly to the bottom, and the exit tube were fused into the neck of the flask. The flasks were thoroughly cleaned and subjected to the vapour of boiling nitric acid for some hours, a necessary precaution to remove any trace of organic matter, as such substances might give rise to CO_2 under bacterial action. All trace of nitric acid was then removed by water condensed in the flasks, this water being derived from the steam of distilled water. Such treatment rendered the flasks perfectly clean, and precluded the slightest fear of any contamination. Into such a flask about 5 grammes of charcoal, freshly heated as previously described, were introduced by means of a clean platinum spatula, the entrance and exit tubes were then plugged with asbestos heated to redness and short pieces of rubber tube fitted to them. The entrance tube was next connected with a flask containing distilled water, and steam from it blown through the research-flask. After enough water had condensed to cover the charcoal and all the air had been expelled from the flask, the apertures were securely closed by clamps while the steam was still passing through. The flask was then allowed to remain for 24 hours, during which time the charcoal would be exposed to a partial vacuum. After this interval air was allowed to enter the flask, the air being passed first through a Mohr's bulb containing strong potash, and then through a short glass tube which was plugged with asbestos freshly heated. The entrance and exit tubes, after disconnection, were again heated to redness, steam passed through, and the flask closed as before. This operation was repeated daily for at least three days. In this manner any gases entangled in the charcoal would be removed, and at the same time complete sterilisation would be effected. Finally the excess of water was removed by evaporation.

Asbestos plugs, previously heated to redness, were always employed in place of cotton wool, as the latter might give rise to CO_2, and thus become a source of error.

Several sets of apparatus, as described, were set up, and into some of the research-flasks bacteria were introduced, while the others were used as controls.

The *bacterium* was obtained from the soil. For the purpose of isolation a number of test-tubes were partially filled with the reheated charcoal moistened in distilled water and sterilised in a steamer. A small quantity of garden soil was shaken up with water, and, after allowing the coarser particles to settle, about 1 c.c. of this water was introduced into one of the test-tubes, which was then placed in an incubator at 20° C. After two days a similar test-tube was inoculated from the first by means of a loop of platinum wire, the process was repeated in a third test-tube, and so on. Those bacteria which could not live on charcoal were thus gradually eliminated, and finally, by constant inspection, a *Diplococcus*, diameter 1 μ, was obtained in pure culture, which was employed for this research. (There is, however, no reason to suppose that this species alone is capable of oxidising carbon, probably it is a property possessed by many other species.)

The bacteria were introduced into some of the research-flasks by removing the asbestos plug and pouring in a little distilled water containing the *Diplococcus.* The aperture was then closed as quickly as possible, the asbestos replaced, and the whole end of the tube heated to dull redness in the Bunsen flame. The inoculated flasks and the controls were then treated in a precisely similar manner. The apertures were closed with rubber tubes and glass stoppers, and all the flasks placed in an incubator at 20° C.; at intervals of a week a stream of air—about 5 litres—was drawn through the apparatus, and titrations were made of the baryta water, great care being taken to prevent the latter from absorbing any CO_2 from the air during this process.

For the first week no CO_2 could be detected in the air from either the controls or the inoculated flasks. This result was not encouraging, and at this stage of the proceedings it appeared as though the investigation might prove fruitless. However, after nearly another week the air contained in the inoculated flasks gave a distinct indication of the presence of CO_2. The amount detected in this way was never large, only amounting to 7 milligrammes per week, but it was measurable and, continuing to be demonstrable while the controls exhibited no trace of this gas, it was sufficient to encourage the further prosecution of the research and the endeavour to confirm the results by other means.

A parallel series of experiments with charcoal in which 5 litres of CO_2-freed air was drawn each morning through the research- and control-flasks gave similar results. In this arrangement the baryta water was contained in Pettenkofer tubes and titrated every seventh day. The control-flasks showed no trace of CO_2, while from the research-flasks, after the first week, an average of 8 milligrammes per week was obtained during a period of one month.

An explanation of this delayed result may be found in the fact that calcium salts are contained in the plant cells from which the charcoal was derived; the calcium oxide therefore present in the charcoal would combine with the CO_2 as soon as formed, and until the process of neutralisation was completed no CO_2 would be present in the stream of air. Also a sufficient time was required for the growth and multiplication of the bacteria.

Determination of Calcium Carbonate.

If the explanation given above were true, calcium carbonate should be present in the inoculated flasks but absent from the controls. Therefore the next step taken was an endeavour to detect the presence of calcium carbonate among the charcoal fragments.

A small portion of charcoal was removed from the inoculated flask and mounted as a microscopic slide; a weak solution of acetic acid was then run under the cover-slip and an evolution of bubbles was immediately seen to take place, while no such evolution could be observed in the charcoal from the controls.

A further confirmation was found in the fact that when freshly-heated charcoal was moistened with water and treated with acetic acid no bubbles appeared, but after exposure for some hours to an atmosphere containing CO_2, a similar treatment with acetic acid resulted in a vigorous evolution of gas.

An objection might be raised that these bubbles were due to the displacement of gases included in the charcoal, and proof is wanting that they were in reality CO_2. This proof was supplied by treating the charcoal in one of the inoculated flasks with weak hydrochloric acid, and then passing a stream of air-free CO_2 through it and then through baryta water. When this was done, barium carbonate was precipitated, and titration showed that 33 milligrammes of CO_2 had been evolved.

A further test in confirmation of the above was afforded by an artificial imitation of the conditions. About 5 grammes of freshly-heated charcoal was exposed to a partial vacuum in the manner before described, in two Cloëz flasks. One of these was filled with CO_2 and the other with air free from CO_2. After standing for 24 hours a stream of air freed from CO_2 was drawn through each of the flasks, and all traces of CO_2 would thus be effectively removed. About 5 c.c. of weak hydrochloric acid was then introduced into each and connections made as speedily as possible with the Reiset absorption apparatus. After the lapse of 24 hours the first flask showed that 34 milligrammes of CO_2 had been given off from the charcoal, and in the second flask only 2 milligrammes, this small amount being possibly due to some residual carbonates which had escaped reduction in the charcoal.

As the analysis of ordinary wood-charcoal gives about 3 per cent. of ash, and lime is one of the chief constituents, the above readings are in agreement with the amount of lime normally present in the charcoal and also with the amount of CO_2 given off from the research-flasks (inoculated with bacteria) when treated with weak hydrochloric acid. This further proves the truth of the supposition that CO_2 is only evolved after the calcium oxide has been converted into the carbonate.

Control experiments made with distilled water inoculated with bacteria, without any charcoal, etc., gave no evolution of CO_2, thus disposing of any criticism which might suppose the carbonic acid to be derived from the bacteria themselves, and not necessarily from their action upon the amorphous carbon. Moreover, the evolution of CO_2 is not confined to the duration of the first experiment, and subsequent titrations made from the same material, which had been returned to the incubators for a further period, invariably demonstrated a further production of CO_2, thus indicating a continuous process of evolution.

Electrical Method of Determining a Rise of Temperature.

Since the phenomenon of oxidation is accompanied by an evolution of heat, it follows that if charcoal were undergoing oxidation it should be possible to detect any rise of temperature due to this process. The amount of CO_2 evolved, however, being small during the period of observation, the rise of temperature to be expected would at most be only a fraction of a degree.

In order to discover whether there was any difference in temperature between the sterile and inoculated charcoal, the apparatus shown in the figure was designed. It consisted of two specially constructed flasks connected with a thermopile and placed in an incubator, with leads passing through a perforation in the side of the incubator to a galvanometer.

Measurement by means of the galvanometer of the E.M.F. produced by two thermo-elements at different temperatures, one placed in a sterile and the other in an inoculated flask, served to determine the difference of temperature between the two.

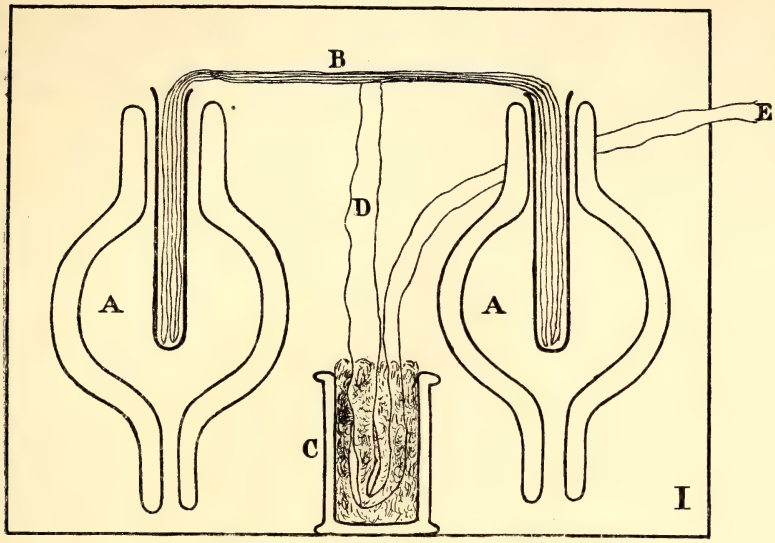


Diagram of Interior of Incubator.

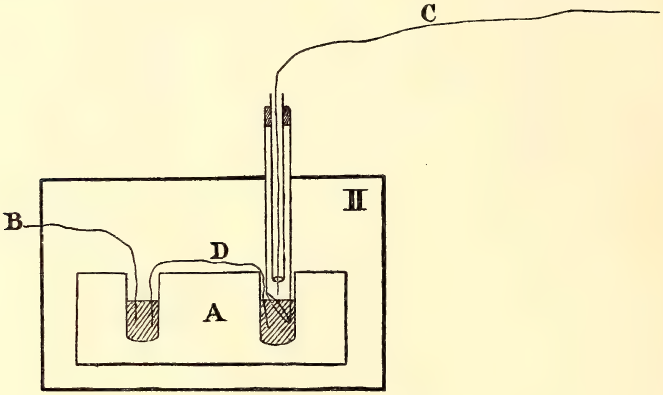
AA, Double-walled vacuum flasks.
B, Thermopile, with terminals inserted in test-tube inside these flasks.
C, Jar packed with cotton-wool, containing junctions of thermopile wires D and galvanometer leads E.

A thermopile with 20 junctions of the iron-nickel combination was employed and formed a highly sensitive instrument. The wires, silk-covered, 32 gauge, had the junctions carefully brazed together and coated with shellac, and the wires themselves were enclosed in thin rubber to give protection against moisture.

The mirror galvanometer used was of the Broca type with a resistance of 50 ohms, and the electromotive force produced by a difference in temperature of 1/200° C. between the terminals registered a deflection of one scale-division. In an experiment of this nature, extreme precautions must be taken with all the junctions of different metals to avoid any thermal effects.

To ensure that the junctions of the thermopile wires and leads to the galvanometer were maintained at the same temperature, these were connected with binding screws, insulated with thin rubber, and packed with cotton wool in a glass jar placed inside the incubator. The galvanometer leads passed through a small perforation in the side of the incubator to a key of special construction and finally to the galvanometer.

A solid block of paraffin formed the basis of the key. Into this block four holes were bored to serve as mercury cups and these were connected in pairs by a metal bridge. One cup of each pair received the wires from the galvanometer and into the others were fixed short glass tubes. Through these tubes the ends of the wires from the incubator could be raised or lowered to break or make a contact. The key was packed with dried cotton wool and enclosed in a box, the two glass tubes slightly projecting through perforations in the lid.



Section of Special Key.

A, Paraffin block with two mercury cups.
B, Lead to galvanometer.
C, Lead from incubator.
D, Connecting bridge of copper wire.

All the junctions of the different metals were thus carefully protected from light or changes of temperature which might set up an electric current, however small, and thus give rise to experimental error.

The accuracy of the measurements of temperature, as recorded by means of the thermopile and galvanometer, is shown by the fact that the calculated differences of temperature were found to correspond with those registered by standard mercurial thermometers under the same conditions.

The Hearson's incubator was first regulated for 20° C., but as this apparatus was heated from one side, it was necessary to ascertain whether the temperature inside was uniform. The terminals of the thermopile were packed with dry cotton wool in glass jars, and placed in different positions inside the incubator, and by this delicate method a difference of temperature of 0°·09 C. was detected between the two sides. On discontinuing the heat and allowing the incubator to assume the laboratory temperature, 14° C., this difference gradually disappeared, and I was able to determine, by renewing the tests, that the air contained within the incubator was then maintained at a strictly uniform temperature. The galvanometer leads passing through the incubator wall obviated the necessity of opening and closing the door, and permitted the research-flasks to remain quite undisturbed.

The glass flasks designed for this experiment were constructed with double walls. The inner flask, with a capacity of about 300 c.c., was prolonged into two tubes diametrically opposite to each other, the upper one serving for the introduction of the charcoal and the lower for the escape of any CO_2 that might be formed. This inner flask was surrounded by a similar larger flask to provide a vacuum, the intermediate space being exhausted of all air in order to prevent any radiation of heat.

Two similar vacuum-flasks were employed. After washing with fuming nitric acid, to remove any organic matter and for the purpose of sterilisation, they were filled with freshly-heated charcoal, which was moistened with sterile distilled water until it had absorbed all the water possible, the surplus being allowed to escape by the lower tube. It is important to note that the flasks were filled from the same supply of distilled water and with the same quantity. Into each of these flasks there was then inserted a thin test-tube previously washed externally in nitric acid and sterilised over a Bunsen flame, and the flasks were plugged with sterile asbestos wool. Finally, the iron-nickel thermopile was introduced into the test-tubes.

The flasks were then placed in the incubator, the connections made with the galvanometer, and the apparatus was complete. Observations were taken at frequent intervals. When first arranged, the unavoidable handling of the metallic junctions necessarily produced thermo-electric currents, but these died away when the apparatus was left undisturbed for two or three days. When on depressing the key no movement of the spot of light could be observed, the whole arrangement was considered to be perfectly reliable, and the experiment could be proceeded with.

One of the flasks was then inoculated with the bacterium, and thereafter readings with the galvanometer were repeatedly taken. At first no deflection was apparent on depressing the key, but after two days a movement of the spot of light could be observed, and by depressing the key synchronically with the swing of the mirror, a deflection of several divisions of the galvanometric scale could be registered. The thermo-electric current gradually increased until after an interval of six days the maximum deflection of 38 divisions was attained, proving a definite rise of temperature in the inoculated flask, while the sterile flask indicated no increase.

To ascertain with certainty that the movement of the mirror was a correct measure of the electromotive force, and in reality due to the difference of temperature between the two flasks, the terminals of the thermopile were interchanged, that is, the terminal in the inoculated flask was placed in the sterile flask and *vice versâ*. The galvanometer then indicated an equal current in the reverse direction (after a sufficient time had elapsed to counteract the effects due to handling), thus proving that the deflection was due to the difference of temperature and not to any accidental error in the apparatus.

By measurement, the maximum deflection indicated by 38 scale-divisions corresponded to a rise in temperature of $0^{\circ}\cdot19$ C., and this temperature was maintained for the further period of a week, when the apparatus was taken down. *This conclusively establishes the fact that a measurable rise of temperature takes place in charcoal, owing to oxidation through the action of bacteria.*

Lamp-black.

Experiments were also undertaken with commercial lamp-black as another source of amorphous carbon, the results of which may be briefly stated. This substance in the first instance was heated to a white heat in the metallurgical furnace; afterwards it was soaked in aqua-regia for nine days, then carefully washed in distilled water, and any excess of acid still remaining neutralised with metallic sodium cut from the centre of a block to ensure the absence of any naphtha. The lamp-black so prepared was treated in the Cloëz flasks in the manner described for charcoal, and some were inoculated with bacteria, while the rest were kept as controls. For a period of 24 days the flasks inoculated with bacteria showed, upon titration, the evolution of a small quantity of CO_2 (17 milligrammes) as compared with the non-inoculated flasks.

The result of the titrations agreeing so closely with those obtained in the case of charcoal, it was not deemed necessary to determine the rise of temperature by the thermo-electric method.

Peat.

A set of experiments upon peat, corresponding to those already described for charcoal, was carried out in an exactly similar manner, except that the peat was not calcined; it will not, therefore, be necessary to do more than briefly state the results. The peat was obtained from the Solway district, and was of the ordinary dried kind such as is used for fuel, *Sphagnum* being the chief constituent.

First, as regards the evolution of CO_2. Several flasks were prepared containing small fragments of the dried peat, soaked with water, and sterilised by discontinuous boiling. A stream of air, carefully freed from all trace of CO_2, drawn through the research-flasks and then through baryta water, failed to exhibit any trace of this gas, even after many days. When this result was well established, some of the research-flasks were inoculated with bacteria, and within a day the cloudy precipitate appearing in the baryta showed that a considerable amount of CO_2 had been evolved as the result of bacterial action, and a copious precipitate continuing to be deposited indicated that a somewhat vigorous oxidation was taking place.

Secondly, to measure any rise of temperature due to oxidation, the experiments with the double-walled vacuum-flasks, the thermopile and galvanometer, were again set in operation, substituting only peat for charcoal. The results were completely in accordance with those noted for the charcoal, except that, as might be expected from the nature of the substance and the consequent greater evolution of CO_2, the oxidation was more vigorous and a greater rise of temperature was recorded. It was found that the inoculated flask maintained a temperature of 1°·05 C. above the incubator for a considerable time.

These experiments clearly show that when peat is exposed to damp air and to the action of suitable organisms it decays rapidly, with the evolution of CO_2, *accompanied by a rise in temperature.*

Coal.

The investigation of ordinary household coal presented many difficulties, chiefly on account of problems connected with sterilisation and the presence of occluded gases, and I have not been able to entirely overcome some special difficulties of the case. Obviously the inflammable gases contained within the coal and its combustible nature rendered sterilising by dry heat an impossibility.

For the purpose of experiment the coal was taken from the centre of a large piece to avoid contamination with foreign matter; it was pounded and passed through sieves similar to those used for the charcoal, and then sterilised in the same manner by passing steam through the Cloëz research-flasks and subjecting the coal to a partial vacuum. The titrations showed that the flasks which were inoculated with bacteria gave off some 10 milligrammes of CO_2 in excess of the non-inoculated flasks during the course of three weeks, but even with the strictest precautions traces of CO_2 were found in the non-inoculated flasks, owing to the escape of this gas from the occluded state. It was this difficulty which first suggested that the measurement of any rise of temperature was a better means for testing oxidation than the collection and determination of the carbonic anhydride.

The thermopile was therefore relied upon in order to determine whether coal sterilised by discontinuous boiling gives out any heat, and whether any thermal changes occur consequent upon the addition of bacteria. Small fragments of coal obtained as described were placed in a flask and immersed in distilled water, the aperture being covered with a small beaker, as cotton wool was inadmissible as a plug. The flask was steamed for five hours on two consecutive days, and on the third day the excess of water was partially driven off by boiling. The coal so treated was then introduced into a sterilised double-walled vacuum-flask, and placed in the incubator, a thermopile was inserted as before, and the connections made with the galvanometer according to the method previously recounted for charcoal. Immediately after setting up the apparatus, the thermopile indicated a temperature of the coal considerably above that of the incubator, this being due to its retaining some heat after boiling; but after 26 hours the research-flask had cooled down to the temperature of the incubator. Following this interval, the temperature of the coal gradually descended, which may be explained by the evaporation continuously taking place from the damp fragments of coal. Then for some three days it remained at 0°·2 below that of the incubator, and it was quite clear that the coal thus sterilised generated no heat.

This point being determined, the coal was inoculated with bacteria by pouring in distilled water containing these organisms, care being taken that the temperature of this added water was below that of the research-flask. The reduction of temperature was at once indicated by the thermopile, the spot of light moving off the galvanometric scale in the direction opposite to the movement when the warm coal was first put in. Twenty-four hours after inoculation the temperature of the research-flask had not only risen to that of the incubator, but it had increased to 0°·08 C. above it, and afterwards a marked rise of temperature amounting to 0°·18 C. was steadily registered for 11 days.

In the experiment just described, one terminal of the thermopile was inserted in the research-flask, and the other in a glass jar packed with cotton wool placed in the incubator, and in this manner any difference of temperature between the research-flask and the incubator could be measured. But the experiment would not be complete without a control, and a second double-walled vacuum-flask, containing fragments of coal from the same source and treated in a precisely similar manner, was also placed in the incubator with a second thermopile, one terminal in each flask. The coal, however, in this second flask was not inoculated with bacteria. Throughout the whole duration of the experiment, the control always maintained a lower temperature (0°·38 C.), than that of the inoculated flask, and by means of a third thermopile it was proved to possess a temperature 0°·19 C. lower than that of the incubator.

For a period of 11 days the temperature of the control flask exhibited no upward tendency, while that of the inoculated flask rose from a point much below that of the incubator to nearly 0°·4 C. above it, and steadily maintained that degree of heat. *It is thus clearly demonstrated that bacteria have a decided action upon coal, resulting in a distinct rise of temperature, and that this increase of temperature does not occur when the coal is preserved from bacterial action.*

Some further experiments with the thermopile and galvanometer upon moist and dry coal emphasise these conclusions. At a temperature of 40° C. the coal moistened with distilled water and inoculated showed a difference of temperature of 1°·25 C. above that of similar coal dried at 100° C. It was also found that at a temperature of 4° C., when the activity of the bacteria would be reduced almost to a minimum, the difference between sterile and non-sterile charcoal was inappreciable, amounting to only 0°·03 C.; while at 14° C. the amount registered was 0°·19 C., which shows that the difference of temperature increases as the thermal conditions become more favourable to bacterial life.*

Important additional evidence that the CO_2 production from the carbon is undoubtedly due to bacterial action would be afforded if it could be shown that the CO_2 is only evolved under conditions consistent with the life of these organisms. I owe to Dr. F. F. Blackman the suggestion that it would be a critical test to show if the CO_2 production increased with a rise of temperature, and whether it goes up or down with causes that have the corresponding effects upon bacterial activity.

With the object of elucidating this point, a further series of experiments was undertaken. Cultures of coal and charcoal were prepared under varying conditions of treatment, and these were subjected to different temperatures of 20°, 30°, 40°, and 100° C. The first three were maintained by Hearson's incubators and the last by a steamer continuously boiling. Cloëz flasks were employed as before, each containing 5 grammes of the research material.

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* In this investigation the actual measurements given must not be understood as representing a quantitative analysis, which would be impossible under the conditions of treatment necessary to preserve the charcoal from contamination. The figures must be regarded only in a qualitative sense, and all that is claimed is that the titration method shows definitely that CO_2 is given off from the research material only when bacteria are present.

From the equation $C+O_2 = CO_2+97650$ calories we learn that 1 milligramme of CO_2 is produced by oxidation, with the evolution of 2·22 calories, and hence the rise of temperature measured by the thermopyle is of the same order as the heat derived from oxidation. But as it is impracticable to suddenly destroy all bacterial action in a vacuum-flask and determine its rate of cooling, an exact equation cannot be obtained.

The conditions selected for the purpose of experiment were:—

(a) *The Provision of Moisture suitable for the Growth of Micro-organisms.*—The charcoal or coal in each flask, after inoculation, was moistened with distilled water, and the flasks then sealed. One of each kind was placed in the steamer, and the others in the incubators at the different temperatures.

(b) *Absolute Dryness which would inhibit Bacterial Life.*—The flasks, after washing, were placed in a drying oven at 100° C., and then heated with the Bunsen flame while a stream of air dried by H_2SO_4 was drawn through them, this operation being repeated several times. The coal and charcoal were also dried for some hours at 100° C., and, while still hot, inserted in the flasks. To remove any trace of CO_2, air was again drawn through them, first passing through a Reiset with a strong solution of caustic soda and then through H_2SO_4. The flasks were sealed as speedily as possible and placed in the incubators.

(c) *Treatment with Antiseptics.*—A 2-per-cent. solution of corrosive sublimate and a solution of iodine in potassic iodide, 95 c.c. of water with 5 c.c. of solution of iodine (12·59 grammes $I + 18$ grammes $KI + 1000$ c.c. H_2O) were the antiseptics preferred, and an excess of chloroform vapour was also employed in deference to the prevalent idea that this substance can be relied upon as an antiseptic. The use of any carbon-compounds, however, such as chloroform or prussic acid, etc., was unsatisfactory, as a special investigation might be required to determine whether they were themselves responsible for any CO_2 production.

The whole series of flasks remained in the steamer and the various incubators for a definite period, after which a stream of air-free CO_2 was drawn through the flasks, then through baryta solution, and titrations made.

The results obtained with uncalcined charcoal are given in the table for the sake of comparison.

The results as set forth in the above table show that under the conditions suitable for the growth of bacteria increasingly higher temperatures indicated a corresponding increase in the amount of CO_2 given off, the production at 40° C. being greatly in excess of that at 20° C.; while at the temperature of 100° C., at which active bacterial life would be impossible, there was no evolution of CO_2. Also, under dry conditions which prevented bacterial growth, no CO_2 was evolved at any of the temperatures tried.

The treatment with antiseptics proved to be untrustworthy and it was evident that this method must be abandoned and the results discarded as valueless for the purposes of the present investigation. There is a danger of chemical reactions taking place, and directly any antiseptic is employed the research becomes complicated by the introduction of an entirely fresh set of problems which need special investigation.

Table of Results of Titrations.

Duration of Experiment 20 Days. 5 grammes of Material used in each case.

	Milligrammes of CO_2.			
Temperatures	20° C.	30° C.	40° C.	100° C.
Coal—				
Moist inoculated	2·0	3·1	4·6	0·0
Moist sterilised by boiling	0·0	0·0	0·0	0·0
Dry	0·0	0·0	0·0	0·0
Charcoal—				
Moist inoculated	0·77	1·1	2·5	0·0
Moist sterilised by boiling	0·0	0·0	0·0	0·0
Dry	0·0	0·0	0·0	0·0
Charcoal uncalcined—				
Moist inoculated	5·4	8·0	22·8	—

Neither corrosive sublimate nor chloroform were found to be effective as antiseptics. In the flasks treated with both these substances a microscopic examination at the conclusion of the experiment showed the presence of motile bacteria (not Brownian movement), which stained with gentian violet and grew feebly as stab-cultures on gelatine. In the flasks treated with iodine no movement of the bacteria could be observed and they appeared to have been entirely destroyed. It should be mentioned that a weaker solution of iodine proved to be less efficient, owing possibly to the smaller margin allowed for its reduction.

I was not prepared to find the bacteria able to resist a 2-per-cent. solution of corrosive sublimate, though chloroform I well knew to be of little use. The value of antiseptics, however, in securing absolutely sterile conditions, is often doubtful, and as the author has previously pointed out, even very strong percentages of such poisons may be quite ineffective in destroying micro-organisms (8 and 9).

The recent work of Adrian J. Brown (2) upon "The Existence of a Semi-permeable Membrane enclosing the Seeds of certain Gramineæ" throws considerable light upon the action of antiseptics. Brown has shown that when these seeds are soaked for three days in a 5-per-cent. solution of cupric sulphate, silver nitrate, and potassium ferrocyanide, no trace of these substances penetrated to the interior of the grain, though water was freely absorbed. After this treatment the vitality of the seeds was not impaired nor their power of germination. Further, the semi-permeable covering enclosing these seeds permits the absorption of water from weak solutions of acids and alkalies, while excluding the latter compounds; but it does not prevent the passage of iodine.

An important question raised by these investigations is : to what extent are plants in general protected by this means, and may it be that some bacteria may possess a cell-wall of a semi-permeable nature which acts as a protective envelope and so accounts for the immunity from injury which many species possess ? Some observations I have made on the behaviour of certain bacteria in solutions of mercuric chloride seem to indicate that this might be so. Plainly, they have some means of protection against a strong solution of such a poison, but it proved too difficult a piece of microscopy to determine whether the sublimate was actually excluded by the enclosing membrane.

The well-known oligodynamic theory of Nägeli (5) may also be cited as bearing upon the effect of antiseptics in certain cases. His observations show that the toxic action of poisons is reduced in the presence of certain insoluble substances such as graphite, etc. True and Oglevee (13) have more recently extended the work in this direction and entirely in favour of Nägeli's conclusions. Their results establish the fact that the presence of certain insoluble substances tends to decrease the toxic activity of solutions of strongly toxic compounds; and their experiments with mercuric chloride prove that the presence of insoluble bodies modifies very markedly the toxic influence of this salt upon the roots of seedlings. The theory advanced is the attraction of the ions, or molecules, of the dissolved substances for the solids and their absorption by the latter.

An explanation is thus afforded of the inefficiency of a high percentage of corrosive sublimate and the behaviour of iodine as an antiseptic, in the presence of finely-divided coal and charcoal. The insoluble nature of the research material, and the possibility of a semi-permeable or selective property of the bacterial cell-wall, appear to offer an intelligible interpretation of the uncertain effect of the antiseptics employed in this research.

The whole question of the dependence upon antiseptics requires consideration, and it is evident that the efficiency of any one cannot be taken for granted, but must be tested for the special conditions under which it is employed.

Lately, Stoklasa (11) has published a preliminary note upon the oxidation of coal and charcoal, and concludes from comparative researches upon "sterile and non-sterile" coal that the evolution of CO_2 is due to: (1) Auto-oxidation, (2) the action of an enzyme. The theory of auto-oxidation is not in accordance with my own experiments, which show that while the amount of CO_2 produced increases with a rise of temperature, CO_2 is not given off at the supra-vital temperature of 100° C., nor under other conditions which prevent the growth of bacteria.

In this preliminary note it is not clear what means Stoklasa employed for sterilisation. If he trusted to corrosive sublimate, the acid reaction commonly found in this salt would have to be taken into account as a source of CO_2 in the presence of any carbonates, as well as other causes affecting its reliability. In some previous experiments Stoklasa (12) himself notes that, notwithstanding all care, active bacteria appeared on the roots of sugar beet which had been steeped for 25 minutes in a 0·5-per-cent. solution of mercuric chloride, and this he attributes to the possible introduction of these organisms in a stream of vapour passed through the apparatus. It seems more probable, as I have proved, that the bacteria flourished in spite of the treatment with mercuric chloride.

Further, Stoklasa does not state whether he re-calcined the charcoal. During re-calcination, various gases are given off, and microscopic examination shows that the cell-wall in ordinary charcoal is often incompletely charred. Thus the combined carbon in ordinary charcoal would be readily attacked by micro-organisms, and on this account the evolution of CO_2 is much greater from uncalcined charcoal than after reheating to about 1200° C.

If the oxidation of carbon takes place through the action of an enzyme, this naturally assumes the presence of a living cell, or, in other words, the oxidation is due, primarily, to bacteria.

General Conclusions.

The methods of experiment which have been dealt with attack the problem from totally different standpoints, and the accumulated evidence affords convincing proof that amorphous carbon slowly undergoes oxidation through the agency of bacteria.

The dependence on antiseptics is shown to be very treacherous, but it is clear that when complete sterilisation is secured by discontinuous boiling, there is no production of CO_2. The results obtained by the experiments with the varying degrees of temperature and under the dry conditions are also of critical importance and establish beyond question my contention that the amounts of CO_2 given off are really due to bacterial activity, and not to any chemical action in the coal or charcoal. If the evolution of CO_2 had proceeded steadily beyond the supra-vital temperature, it would have pointed to a non-vital change, but the fall of CO_2 at the death point clearly indicated the cessation of a vital process.

The determination, by measurement with the thermopile, of the rise of temperature due to oxidation becomes a very valuable confirmatory experiment in conjunction with the method of testing the evolution of CO_2 by titration.

It has been conclusively established by means of the thermopile that a definite rise of temperature occurs when carbonaceous substances such as charcoal, coal, peat, etc., are subject to the action of certain bacteria. And in this connection it must be remembered that the rise of temperature is maintained for some considerable time, and that the double-walled vacuum-flask, with at least a portion of the wires composing the thermopile, are steadily preserved at this temperature above the surrounding medium, in spite of any loss from radiation. There must thus be a continuous dissipation of heat and the amount generated is therefore more than actually appears; it must also be taken into consideration that this rate only applies to conditions *in vitro* and in a laboratory. Probably, in the soil, the carbon would be attacked by micro-organisms under circumstances more favourable to their activity and the oxidation would proceed much more rapidly. It is of importance to recognise that every process of oxidation raises the temperature in an appreciable degree, and this is a factor which should be taken into account in all problems relating to the soil.

Indeed, the action of bacteria in promoting exothermal changes is a subject too generally neglected. It must now be recognised as possessing a practical bearing upon investigations connected with oxidation of coal. The suggestion may also be made that in some cases of spontaneous combustion of coal, the heat generated by microbial activity is an influence to be taken into consideration, and may be a dangerous motive force acting upon explosive gases.

That carbon should be proved to undergo oxidation by bacteria is not surprising when we consider the fact that nitrogen undergoes the same process, while the oxidation of sulphur by bacteria has been established by Beijerinck (1) and quite recently that of hydrogen by Kaserer (4). The author has previously shown that oxalic acid undergoes decomposition into CO_2 and H_2O by the agency of a soil bacterium (6), a result which has since been confirmed independently by Hall (3).

Incidentally the present investigation throws some light upon the formation and decomposition of coal. In the ordinary course of events vegetable matter gradually undergoes a process of decay, countless bacteria and fungi deriving their sustenance from it and gradually effecting the dissolution of the cellulose and other compounds, until, ultimately, the end-products are reached. This process demands, among other things, a constant supply of oxygen, and in the absence of this element only a partial reduction can be attained. This is well exemplified in the case of peat, where the vegetable *débris* in a wet and sodden condition is excluded from a sufficient supply of oxygen, and therefore the oxidising organisms are unable to carry on their work, any further changes must be due to anaerobic forms, and the decay is necessarily incomplete. In a similar manner it may be supposed that the large deposits which form the basis of coal are due to plant remains which, in the first instance, were precluded from complete oxidation owing to their submerged situation, where vegetable matter could only be acted upon by anaerobic bacteria.

In this connection, Renault's (10) researches upon Fossil Bacteria are of great interest. He brings forward evidence to show that bacteria have existed since Devonian times, and have played a considerable part in the destruction and decomposition of vegetable and animal tissues from this remote period. Microscopic sections have furnished remarkable proof of their presence in the upper Jurassic beds, in the Permian Strata, the Upper, Middle, and Lower Coal Measures, in the Carboniferous Limestone, and in the Devonian, and these illustrate in a remarkable manner the destructive action of numerous micro-organisms upon organic remains imbedded in these formations. Renault states that in many cases the minutest details have been preserved in such perfection that it has been possible to detect the bacteria often more easily in the fossilised than in the living state. All stages in the disintegration of the cell-tissues are clearly exhibited, and it is proved that the *rôle* of these micro-organisms has been identical with that which they perform in the present day.

According to Renault, "If in the formation of coal there are two distinct phases, one, purely chemical, which has brought the remains of plants to a certain composition answering roughly, in the case of 'houille de bois pur,' to the formula $C_{10}H_8O$, the second, simply mechanical, due to a slow compression in a permeable medium, the first of these phases can be attributed to a bacterial fermentation developed in the marshes, ponds, deltas, and arrested by periodic floods, carrying away a portion of the macerated plants and transporting them into lakes and seas, where maceration became impossible." In later times heat and pressure would convert this partially decayed vegetable *débris* into coal. The decay has, however, only been arrested, and, like peat, when a sufficiency of oxygen is available and the necessary conditions for the life of aerobic organisms are presented, the decomposition proceeds, the elements are reduced to their simplest compounds, and the carbon is once more liberated in the form of CO_2 to play its *rôle* in the life cycle.

Coal and peat are shown to be subject to the same laws as other organic matter, and such substances remain unsusceptible to change only so long as bacterial action is excluded.

It is quite evident that where there is surface exposure bacteria must play a large part in the disintegration of coal, which is one of the most insoluble substances known, and that these organisms form an invaluable agency in assisting the circulation of carbon and again converting it to the uses of Nature.

Summary.

Under conditions of exposure to the air, a slow oxidation of amorphous carbon takes place through the agency of bacteria. This has been conclusively established by experiments upon such carbonaceous substances as charcoal, lamp-black, coal, and peat.

When these substances are subjected to bacterial action carbonic acid is given off, as estimated volumetrically by absorption in baryta solution and titration with standard oxalic and hydrochloric acids.

The amount of CO_2 given off increases in proportion to the rise of temperature and ceases to be evolved at a supra-vital temperature. There is no evolution of CO_2 under perfectly dry conditions such as preclude the possibility of bacterial life.

A distinct rise of temperature occurs through the action of bacteria. The heat generated was determined by measurement, with a galvanometer, of the electromotive force produced by the difference of temperature between two thermo-elements, one placed in a sterile and the other in an inoculated flask.

The evolution of CO_2 and the accompanying rise of temperature does not take place when carbonaceous substances are preserved from the intrusion of micro-organisms.

The heat generated by microbial activity is an influence to be taken into account in connection with the oxidation and spontaneous combustion of coal; it may be a dangerous motive force acting upon explosive gases.

The oxidising action of bacteria must be largely responsible for the disintegration of coal and the high percentage of depreciation which it undergoes in store.

Coal and peat, like other organic matter, are liable to decomposition as soon as conditions are presented suitable for the life of aerobic organisms. The carbon is then once more liberated in the form of CO_2 to play its *rôle* in the life cycle. It is thus conceivable that the vast supplies of carbon locked up in the world's coal-fields may become available for plant nutrition without the intervention of direct combustion.

I have to express my indebtedness to my colleague, Dr. Morris-Airey, for much help which he has given me in the preparation and testing of the electrical apparatus employed in this research, and I take this opportunity of thanking him for the kind way in which he has always been ready to give me the benefit of his assistance in any technical difficulty.

My obligations are due to the Government Grant Committee of the Royal Society for the use of a Zeiss 3 mm., 1·40 apochromatic oil-immersion lens.

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13. True and Oglevee, "The Effect of the Presence of Insoluble Substances on the Toxic Action of Poisons," 'Botanical Gazette,' vol. 39, 1905.

The Antagonistic Action of Calcium upon the Inhibitory Effect of Magnesium.

By S. J. Meltzer and John Auer.

(Communicated by Professor E. H. Starling, F.R.S. Received March 10,—
Read April 2, 1908.)

(From the Department of Physiology and Pharmacology of the Rockefeller Institute for Medical Research.)

Calcium and magnesium are chemically closely related elements. They are also close companions in the tissues of the animal body. It is the prevailing view that the physiological effects of both elements are similar in character. Many physiologists are at present of the opinion that calcium as well as magnesium exerts an inhibitory influence in the functions of the animal body. Loeb published, in 1899, his observations of the inhibitory action of calcium upon the twitchings of the frog muscles brought on by solutions of sodium chloride.* It was then assumed by Loeb that all the members of the group of alkali earths possess inhibitory properties, including, at first, even barium. In the numerous subsequent papers by Loeb and his pupils, the discussion turned, however, essentially around the inhibitory effect of calcium.

As to magnesium, we have within the last few years published several studies in support of the hypothesis that magnesium salts favour inhibitory processes. The first fact which gave rise to that hypothesis was demonstrated in 1899 to the American Physiological Society, when an intracerebral injection of a few drops of a solution of magnesium sulphate caused a state of paralysis in a rabbit, while the injection of other solutions brought on convulsions.

In a series of recent studies which we have carried out upon the relations of the effects of calcium to magnesium, many facts came to light which demonstrate unmistakably that calcium is the most available agent to neutralise inhibitory effects of magnesium. We shall not enter here upon details; we wish only to report the following striking and instructive experiment.

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* (*Note by E. H. Starling.*—The inhibitory action of calcium salts on the twitching brought on by sodium chloride solutions was observed by Dr. Ringer, F.R.S., many years before Loeb, and is fully described by him in a paper in the 'Journal of Physiology,' vol. 7, p. 291, 1886. In reference to the subject of the present communication, it is interesting to note that Ringer observed a similar antagonism between barium and calcium (*vide* 'Practitioner,' vol. 31, p. 81, 1897).)

By subcutaneous injections of a magnesium salt (for instance, Epsom salt—about 7 c.c. of a 25-per-cent. solution per kilogramme) rabbits are brought to a profound state of anæsthesia and paralysis. The slow and shallow respirations indicate the approaching danger. Now 6 or 8 c.c. of an M/6 or an M/8 solution of a calcium salt are given through the ear vein. Within a few seconds the respiration becomes quicker and deeper, and within one minute the animal turns over, sits up, and appears normal.

Here calcium not only did not add an inhibitory effect, but completely neutralised the profound inhibitory effect of magnesium. The companionship of calcium and magnesium within the body means, at least in many instances, not a concerted action of similar effects, but rather a resultant effect of antagonistic actions.

We may add that the experiment calls to mind similar relations existing in plant physiology; the retardation of growth on account of the presence of too much magnesium in the soil is promptly corrected by the addition of a calcium salt; the process is termed "liming." In animals, therefore, as well as in plants, calcium is antagonistic to magnesium.

Post-tetanic Tremor. (*Supplementary Note.*)

By David Fraser Harris, M.D., B.Sc. (London).*

Since the publication of my paper on this subject,† my attention has been called to the fact that the phenomenon has been previously noticed by Dr. Sydney Ringer.‡

I regret that I was unacquainted with his paper until after mine had been published. Dr. Ringer states:—"This powerful prolonged faradisation, for one or two minutes, of the sciatic nerve of a cut-off leg of an undrugged frog, causes the limb to remain extended, and if it be held vertically, foot upwards, it falls more slowly than happens after only a momentary stimulation"; and again: "These fibrillary twitchings and this spastic condition can be produced in normal imprisoned muscle." There can be no doubt that what Dr. Ringer called "fibrillary twitchings" and I have termed "post-tetanic tremor" are identical phenomena.

In connection with the tremor which occurs in the muscles of the lobster during faradic stimulation, I ought also to have alluded to Professor C. Richet's discovery§ of an identical tremor ("tetanos rythmique") in the pincer muscle of the crayfish, but I was unable to obtain a copy of his paper until after my own had gone to press.

[* This supplementary note was received March 11, 1908.—Sec. R.S.]

† 'Roy. Soc. Proc.,' B, vol. 80, p. 37, 1908.

‡ "Report on the Influence of Rhombic Sodium-phosphate and Sodium-bicarbonate on Muscular Contraction," 'Brit. Med. Journ.,' July 19, 1884, vol. 2, p. 114.

§ "Contribution à la Physiologie des Centres Nerveux et des Muscles de l'Écrevisse," 'Arch. de Physiologie Norm. et Path.,' fig. 22, p. 563, vol. 6, 1879.

The Glycogenic Changes in the Placenta and the Fœtus of the Pregnant Rabbit: a Contribution to the Chemistry of Growth.

By J. Lochhead, M.A., M.D., B.Sc. (Carnegie Scholar), and W. Cramer, Ph.D., D.Sc. (Lecturer on Physiological Chemistry, University of Edinburgh).

(Communicated by E. A. Schäfer, F.R.S. Received January 1,—
Read February 27, 1908.)

(From the Physiology Department, University of Edinburgh.)

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Introduction.

Although a special formative power has been assigned to glycogen ever since the widespread distribution of this substance in the growing tissues of embryos became known, no systematic quantitative investigations into the glycogenic changes of the embryo have yet been made. The histological observations of Maximow* and of Chipman† on the placenta of the rabbit showed that glycogen appears in the walls of the maternal placenta at about the 8th day of pregnancy, and increases in amount until a maximum is reached at the 12—16th day. After mid-term the glycogen diminishes, and completely disappears at the end of gestation from that part of the maternal placenta which is next to the fœtal part of the placenta. In the fœtal placenta no glycogen could be demonstrated histologically.

In Ruminants, conditions differ entirely from the rabbit. Bernard himself, in investigations extending over several years, failed to find glycogen in the placentæ of sheep and cows. He did, however, find it in the flattened or papilliform bodies with which the umbilical cord and the internal surface of the amnion are covered, the quantity increasing up to mid-term and then decreasing.

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* Maximow, 'Zeitschrift für Mikroskopische Anatomie,' vol. 51, 1898, p. 68.

† Chipman, 'Laboratory Report Royal College of Physicians, Edinburgh,' vol. 8.

Lately Jenkinson* has again investigated sheep's placentæ histologically and found glycogen in the uterine epithelium and trophoblast of the extra-cotyledonary region; there was none apparently in the maternal cotyledons and none in the villi.

The time of origin of the glycogenic function of the liver has long been a subject of controversy. While Bernard† and Barfurth‡ concluded from their investigations that the liver first functioned as a glycogen-secreting organ about the middle of intra-uterine life, Pflüger§ proved by means of his more reliable method that small quantities of glycogen are present in the liver at much earlier stages. According to this author, the fœtal liver shows the same behaviour with regard to its glycogenic function as the adult liver, and since his material was obtained from the slaughter-house, he ascribed the small amount of glycogen found in the fœtal liver to the imperfect feeding of the mother animals before they are slaughtered.

The recent work of Lubarsch,‖ Gierke,¶ Adamoff,** and especially the detailed chemical investigation of Mendel and Leavenworth,†† have proved that fœtal tissues are not so rich in glycogen as adult tissues, as was believed formerly. While the earlier observers concluded from their observations that glycogen was an essential constituent of tissues in which a rapid cell formation and cell development take place, the later authors deny that there is any relation between the energy of growth of embryonic tissues and their glycogen metabolism.

The present investigation is an attempt to throw light on the part played by glycogen in the development of the fœtus by means of a systematic investigation on the glycogenic changes in an age-series of fœtal animals.

Methods.

For this investigation rabbits were used. The animals were kept in the laboratory, and the date of insemination was noted in each case. In this way it was possible to obtain an accurate and almost complete age-series from the 14th day to the end of pregnancy.

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* Jenkinson, 'Proceedings Zoological Soc. of London,' vol. 1, 1906.

† Bernard, 'Journal de la Physiologie de l'Homme,' vol. 2, 1859, p. 335.

‡ Barfurth, 'Archiv für Mikroskopische Anatomie,' vol. 25, 1885, p. 529.

§ Pflüger, 'Pflüger's Archiv,' vol. 102, 1904, p. 305.

‖ Lubarsch, 'Archiv für Pathologische Anatomie,' vol. 183, 1906, p. 192.

¶ Gierke, 'Glycogen in der Morphologie des Zellstoffwechsels,' Habilitationsschrift. Freiburg, 1905.

** Adamoff, 'Zeitschrift für Biologie,' vol. 46, 1905, p. 281.

†† Mendel and Leavenworth, 'American Journal of Physiology,' vol. 20, 1907, p. 107.

As a rule, the material for such investigations is obtained from the slaughter-house, and the age of a fœtus is determined by its size or by its weight. Under normal conditions this is a perfectly reliable method, and material can be obtained much more easily in this way; but it does not take into account the possible occurrence of conditions in which the fœtuses have an abnormally low weight or an abnormally small size. It will be seen that we have met such conditions, and that they have given us interesting and suggestive results, which could not have been obtained otherwise.

The material on which our observations were made is given in the following table.

Table I.—Material.

Day of gestation	14.	16.	18.	20.	21.	22.	23.	24.	25.	26.	27.	28.	29.
Number of fœtuses	5	8	8	6	5	4	8	6	7	5	3	6	9

For the estimation of glycogen, Pflüger's method* was used, and the special precautions recommended by him for the analysis of small organs were carefully attended to.† The maternal and fœtal parts of the placenta, which admit of fairly accurate mechanical separation in the rabbit, were analysed separately. In the fœtus the glycogen was determined in the liver and in the rest of the body respectively. The method of procedure was as follows:—

Immediately after death the abdomen of the pregnant animal was opened and the whole uterus removed. Each sac was opened along its convex border and the fœtus extracted. Then the fœtal placenta was gently pulled asunder from the maternal and the membranes were carefully cut away from it. Finally the maternal placenta was cut away from the uterine wall with scissors, a method which included the zone of separation with its glycogen-containing cells in the part for analysis. In the later stages of pregnancy, the maternal placenta was detached from the uterus, and the two parts of the placenta had then to be separated from each other. In the rabbit there is no intergrowth between maternal and fœtal tissues, but the mechanical separation is not quite perfect, some of the maternal peninsulæ that extend down between the fœtal columns being left attached to the fœtal placenta. All the maternal placentæ were then weighed and afterwards placed together in a 100-c.c. flask, to which a heated solution of caustic potash (60 per cent.) was added—1 c.c. of the alkali for each gramme of tissue—and kept in a boiling water bath for some hours. The same was done with the fœtal placentæ. The average weights of these two tissues per fœtus may be seen in Tables II and III. Then the fœtal livers were excised, care being taken not to include any of the intestine, and to empty the gall-bladder, which was always distended with a viscid greenish-yellow fluid. The livers and the remainder of the fœtal bodies were weighed, and each set placed in a stoppered flask of known capacity and treated like the maternal placentæ. Each tissue was liquefied by the strong alkali, except the fœtal bones. The rest of the process was carried out in every respect as Pflüger recommends.

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* Pflüger, 'Pflüger's Archiv,' vol. 93, 1903, p. 163.

† Pflüger, 'Pflüger's Archiv,' vol. 111, 1906, p. 307.

The results are set down in tabular form in Tables II to V. As the number of placentæ and fœtuses varied, it was necessary for purposes of comparison to calculate the results per placenta and fœtus in each case. It may be pointed out that the amount of glycogen given in these tables does not represent the total amount of glycogen actually obtained in each analysis. This can be found by multiplying the amount given for each case with the number of fœtuses for that day given in Table I.

A. *Glycogen of Placenta.*

For a better understanding of the results, it is necessary to give a brief account of the anatomical plan of the rabbit's placenta with its glycogen areas according to Chipman. In the maternal placenta, three parts can be recognised:—

(1) That next the uterine muscle which represents the plane of separation of the placenta at the end of gestation;

(2) An intermediate part, the region of the uterine sinuses; and

(3) A part which extends up as a series of peninsulæ between the fœtal columns. These columns are analogous to the villi of the human placenta, and are described by Chipman as ectodermic tubules with a plasmodial covering.

Glycogen Areas.—In the maternal placenta, the glycogen is always contained in decidual cells, and three glycogen areas can be distinguished.

(A) At the zone of separation. A definite quantity of glycogen is still present in this part at the end of gestation.

(B) In the region of the uterine sinuses lying in the multinucleate decidual cells surrounding them. Here there is probably no glycogen left at the end of pregnancy.

(C) In the peninsular projections. This is the part most intimately associated with the fœtal tubules, and, indeed, these projections are entirely covered by fœtal ectoderm.

When the two parts of the placenta are pulled apart, the delicate peninsulæ are left attached to the fœtal placenta. In the latter no glycogen was observed by Chipman at any period of pregnancy. Hence the glycogen obtained from the fœtal placenta is not, as might appear at first sight, actually in the fœtal tissue, but really belongs to the maternal placenta, and forms that part most intimately related to the fœtus.

By analysing the two parts of the placenta obtained by mechanical separation, information is gained on the changes in the glycogen of two different parts of the maternal placenta. The bulk of the maternal placenta contains the glycogen of the zone of separation (A) and of the region of the uterine sinuses (B). This part of the glycogen may be called for convenience the "distal glycogen." The glycogen in the peninsular projections (C), which are left attached to the fœtal placenta after mechanical separation, may be called "proximal glycogen." It is estimated by analysing the fœtal placenta, as no glycogen can be demonstrated histologically in the fœtal placenta itself.

It is possible that a small part of the glycogen extracted from the fœtal placentæ in this way may actually belong to the cells of the fœtal tissue, as the villi, like the early fœtal liver, may possess a small quantity of glycogen not demonstrable histologically. The colour reaction with iodine was tested in several cases, because it has been found that the tint is violet-red with glycogen obtained from fœtal tissues instead of the usual reddish-brown. In no case was there any difference from the colour obtained with maternal glycogen. Hence most of the carbohydrate extracted from the fœtal placenta was probably maternal. We shall see later that very small quantities of glycogen may be present even when histological methods fail to reveal it, but in any case the amount could not be large enough to affect materially the results obtained.

The results obtained from the analysis of parts A and B of the maternal placenta are given in Table II.

The maternal placenta has, in every case, a considerable percentage of distal glycogen, and one that is in the earlier stages quite comparable with the percentage obtained in a healthy adult rabbit's liver. It rises from the 14th to the 18th day. From the 18th to the 22nd day it remains fairly constant; then there is a continuous decrease each day until the end of gestation. Even at the 29th day there is an appreciable amount of glycogen, which, as histological examination shows, is almost entirely situated at the zone of separation of the placental lobes, and is probably not destined for the fœtus.

The most obvious phenomenon in the decidual cells of the rabbit is the presence of glycogen at a time when the fœtal liver cells store only the

Table II.—Glycogen and Weight of Maternal Placenta (Distal Part).

Day of gestation	14.	16.	18.	20.	21.	22.
Average weight of maternal placenta in grammes	0·72	0·51	1·30	1·60	1·34	1·73
Average weight of glycogen per placenta in grammes	0·0320	0·0247	0·0729	0·0858	0·0746	0·0931
Glycogen percentage.........	4·44	4·81	5·66	5·36	5·57	5·40

Day of gestation	23.	24.	25.	26.	27.	28.	29.
Average weight of maternal placenta in grammes	1·63	1·63	1·41	1·40	1·23	1·08	0·56
Average weight of glycogen per placenta in grammes	0·0731	0·0733	0·0515	0·0392	0·0316	0·0162	0·0065
Glycogen percentage	4·50	4·49	3·64	2·80	2·56	1·50	1·18

merest traces. But in the last week of pregnancy the liver carries out this function for the fœtus, while the placental glycogen gradually diminishes, and simultaneously with this the decidual tissue degenerates and is absorbed. It is this connection between the healthiness of the decidual cells and their store of glycogen, on the one hand, and their degeneration after the glycogen store has been absorbed by the syncytium, on the other, which leads us to suppose that they have a definite function to fulfil during pregnancy, and do not merely exist, as is usually stated, as pabulum to be absorbed by the syncytium.

A remarkable feature of the results is the absence of individual variations.

The changes in the "proximal glycogen" are tabulated in Table III, which gives the results of the analyses of the fœtal placentæ with the peninsulæ of the maternal placenta attached. It is obvious that in this case no information is obtained by expressing the "proximal glycogen" belonging to the maternal placenta in terms of percentage of the fœtal tissue, which is mainly fœtal in origin and the weight of which may, in fact, be taken as indicating the weight of the fœtal placenta. It is worthy of note that the weight of the fœtal placenta does not show a decrease in the last week of pregnancy like that seen in the maternal placenta.

The variations in the proximal glycogen follow approximately those observed in the distal glycogen. On the 29th day there is no glycogen remaining in this part of the placenta. But up to a period within 48 hours of labour there is still glycogen present in a suitable position for absorption by the fœtal tissues.

Table III.—Glycogen of Maternal Placenta (Proximal Part). Weight of Fœtal Placenta.

Day of gestation	14.	16.	18.	20.	21.	22.	23.	24.	25.	26.	27.	28.	29.
Average weight of fœtal placenta in grammes	0·26	0·24	1·03	1·40	1·90	2·30	2·38	2·67	2·83	1·90	3·93	2·70	2·72
Average weight of glycogen per placenta in grammes	0·0040	0·0032	0·0072	0·0102	0·0110	0·0082	0·0062	0·0043	0·0017	0·0011	0·0016	0·0008	0·00

B. *Glycogen of Fœtal Liver.*

Table IV shows the results of the analyses of the fœtal livers from the 18th day onwards.

Table IV.—Glycogen and Weight of Fœtal Liver.

Day of gestation	14.	16.	18.	20.	21.	22.	23.	24.	25.	26.	27.	28.	29.
Average weight of fœtal liver in grammes	Too small for analysis		0·09	0·42	0·54	0·68	0·70	1·0	2·03	0·74	2·67	2·07	2·11
Average weight of glycogen per liver in grammes			Trace	0·0002	0·0003	0·0006	0·0013	0·0026	0·0116	0·0016	0·0392	0·0703	0·0536
Glycogen percentage.........			0·00	0·04	0·06	0·09	0·19	0·25	0·57	0·21	1·47	3·40	2·54

In every case glycogen was present, though in the earlier stages the amounts were extremely small. Thus, at the 18th day no quantitative estimation was attempted, but the iodine reaction was positive, and the extract, after inversion with acid, reduced a small amount of Fehling's solution. Up to the 21st or 22nd day the amounts present were too small for accurate quantitative estimation, but the results are in all cases approximate. It is interesting to note that glycogen cannot be demonstrated histochemically in the fœtal liver before the 22nd day, and the percentage then is about 0·1.

Up to the 24th day the amount of glycogen is very small, and a comparison with Table V shows that the percentage is below that of the remainder of the fœtal body. Pflüger also obtained very small amounts in the liver of early fœtuses, and sometimes had to be content with the qualitative demonstration of glycogen by the iodine reaction, even in organs weighing a considerable number of grammes. At the same time he found the fœtal muscles to possess appreciable amounts, and he seeks to explain this anomaly by the imperfect feeding of the mother animals (sheep) for some days before they are slaughtered. The same objection might also be urged against some analyses we made of early fœtal livers of sheep which showed only traces of glycogen. But such an objection is inadmissible for our series of fœtal rabbits, in which the pregnant animals were kept on full diet up to the time of death. We must conclude, therefore, that during the earlier stages of development the liver contains only traces of glycogen, and has not yet taken up its glycogenic function.

After the 24th day the analyses show different results. At the 25th day the percentage of glycogen rises, for the first time, above that of the remainder of the fœtal body, and on each subsequent day there is a well-marked increase, till at the 29th day we find almost half of the total fœtal glycogen stored in the liver. Hence, though glycogen is present as early as the 18th day at least, a marked change takes place in the fœtal liver about the beginning of the last week of pregnancy. It is only then that the liver takes up its glycogenic function. This fact accounts for the conflicting results which have been obtained by various authors from isolated observations on the glycogen content of fœtal livers.*

The results in no case reached the percentages obtained in maternal livers, which varied between 4·1 and 7·4 per cent., while the highest figure obtained in a fœtal liver was 3·4 per cent. An exceptionally low result was obtained in the case of the animal killed on the 26th day. This animal, however, was in an abnormal state; one fœtus was dead and the others were smaller than usual. The weight and glycogen percentage of the maternal placentæ were about normal, but the fœtal placentæ were small; the fœtal livers weighed much less than might have been expected, and the glycogen percentages of fœtal bodies and fœtal livers were both low. The animal killed on the 29th day of pregnancy also gave results slightly below the normal.

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* See Demant, 'Zeitschrift für Physiolog. Chemie,' vol. 11, 1887, p. 142.

C. *Relationship of Placental to Fœtal Liver Glycogen.*

The relationship between the glycogen percentage of the placenta and fœtal liver is shown in graphic form in fig. 1. For the former, the total

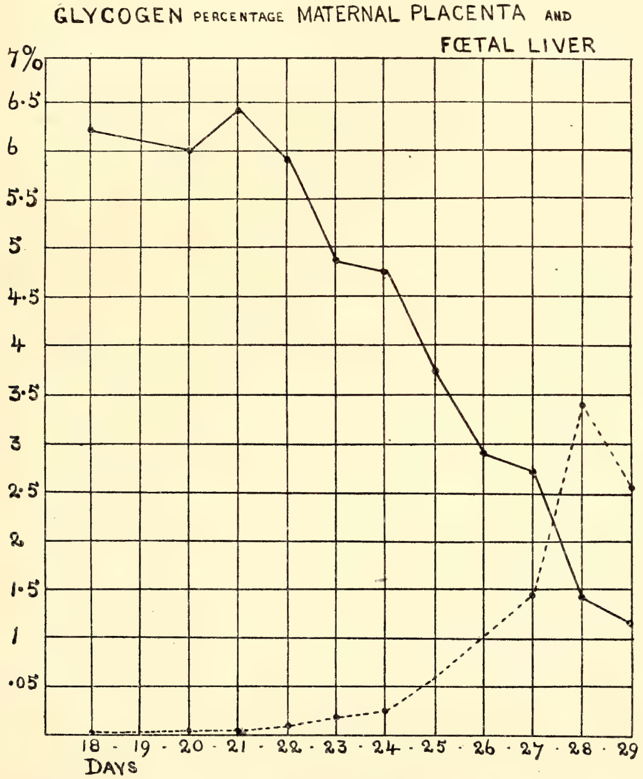

Fig. 1.—The abscissa represents the period of gestation from the 18th day onward. The ordinate gives the glycogen percentage. The glycogenic changes in the placenta are represented by the black line, those in the fœtal liver by the dotted line.

placental glycogen was taken, *i.e.*, the sum of the amounts found in the maternal and fœtal parts of the placenta, and its percentage value was calculated from the weight of the maternal placenta. The figure shows how closely the decrease of the percentage in the one organ is related to the increase of the percentage in the other. This relation is so striking that one is led to conclude that the placenta carries out in the earlier stages of pregnancy the glycogenic function for the fœtus which is fulfilled later by the fœtal liver.

D. *Glycogen of Rest of Fœtal Bodies.*

Table V shows the results obtained from the analyses of the fœtal bodies after the liver had been removed.

Table V.—Glycogen and Weight of Remainder of Fœtal Body.

Day of gestation	14.	16.	18.	20.	21.	22.	23.	24.	25.	26.	27.	28.	29.
Average weight of rest of fœtal body in grammes	Too small for analysis		0·8	1·90	2·74	3·45	6·50	8·75	18·20	10·50	30·17	30·00	24·56
Average weight of glycogen per fœtus in grammes			0·0018	0·0048	0·0077	0·0097	0·0190	0·0320	0·0692	0·0241	0·1026	0·1314	0·0663
Glycogen percentage			0·23	0·25	0·28	0·28	0·29	0·37	0·37	0·23	0·34	0·44	0·27

The great increase in the total amount of glycogen per fœtus is, of course, to be expected along with the great increase in weight as pregnancy advances. The percentage amount is also higher in the last week of pregnancy than in the period immediately preceding, but the variation is only slight. The results agree with the work of other investigators in showing that embryonic tissues are not particularly rich in glycogen.

Relation of Fœtal Growth to Glycogen Percentage.—Fig. 2 represents graphically the increase in fœtal weight and the increase in the percentage of the total fœtal glycogen from the 18th day to the end of gestation, as calculated from Tables IV and V. As is to be expected, the curve of the fœtal growth rises as pregnancy advances. Corresponding to this we have a general rise, not only in the total amount of glycogen, but also in the glycogen percentage of the fœtus. In the two exceptional cases where the fœtal weight was lower than that of the preceding day there was also a decrease, not only in the absolute amount of glycogen, but also in the percentage; i.e., the glycogen was decreased out of proportion.

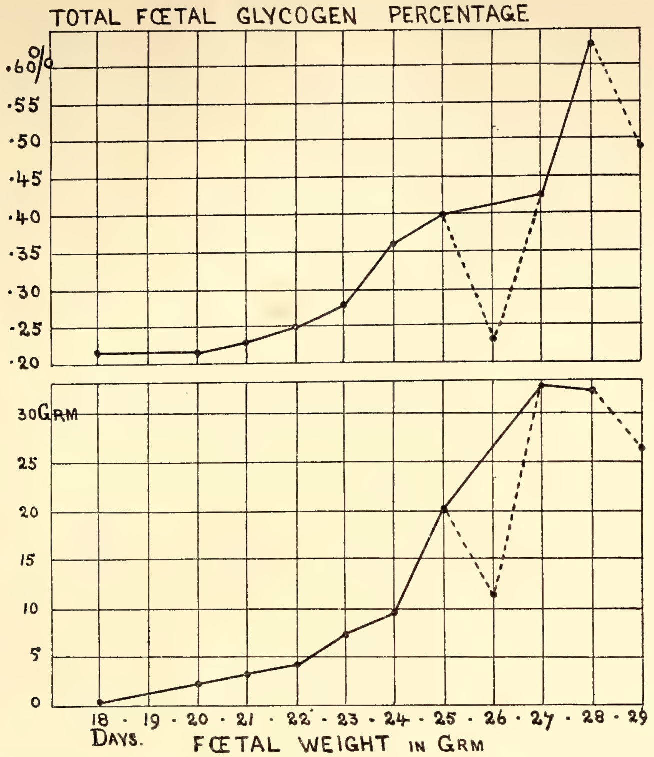

FIG. 2.—Both curves have the same abscissæ indicating the days of gestation from the 18th day onward. The ordinates of the upper curve represent the glycogen percentage of the fœtal organism from 0·20 to 0·60 per cent. The ordinates of the lower curve represent the weight of the fœtus in grammes from 0 to 30 grammes. The dotted lines indicate the two abnormal cases referred to in the text.

There is, therefore, a distinct parallelism between the growth of the fœtus and the percentage amount of glycogen which it contains. As we have to deal here with an organism in which the life and growth of each single cell is not autonomous, but is dependent upon the metabolism of the whole, such a relation does not necessarily imply, as has been commonly held, that the quickly growing cells themselves are especially rich in glycogen. Indeed, this is, as we have seen, not the case. But if, instead of considering a single cell, we look at the fœtal organism as a whole, a distinct interdependence between the growth of the fœtus and the glycogen present in the organism becomes apparent.

Attempts were made, by variations in diet and by injection of phloridzin, to alter the glycogen percentage of the placenta, in order to find out if a corresponding change could be produced thereby in the fœtus.

Effect of Variation in Diet.—The results of the feeding experiments on the placenta, both maternal and fœtal, are given in Table VI. Rabbit A was fed on a diet rich in carbohydrates for 24 hours before it was killed. Six others were fed on a similar diet from the beginning of pregnancy, but results were only obtained in three (Rabbits B, C, and D), as the remaining three aborted about mid-term.

In addition, in these experiments the liver glycogen of the mother animals was also determined. Burlando* has recently stated that in dogs the liver glycogen increases markedly during pregnancy; but this is not borne out here. The percentages are no higher than those commonly found after such a diet in non-pregnant animals.

With each the control from the normal series is given.

The results show a remarkable correspondence with those obtained on ordinary feeding. There is no increase in the placental store of glycogen as the result of a diet rich in carbohydrate.

The amount of glycogen present in the placenta appears to be independent of the conditions of diet and regulated entirely by the needs of the fœtus, a fact which is of importance in the development of the embryo. Nor has feeding with an excess of carbohydrates any effect on the date at which the fœtal liver assumes its glycogenic function or on the amount stored in the later stages. The glycogen percentage of the rest of the fœtal bodies and the weight of the fœtus were also not affected by the variation of diet.

The results obtained in this series corroborate those of the more complete age-series examined under normal conditions. The constancy in the amount of glycogen deposited in the placenta and in the fœtal tissues at the various periods of fœtal life contrasts markedly with the fluctuations in the glycogen store of the adult liver, both in the normal and, as our figures show, in the pregnant animal, and demonstrates again the autonomy of the glycogen metabolism of the fœtus.

E. *Effect of Injections of Phloridzin.*

In order to induce experimentally a diminution of the glycogen store, and in this way to study further the relation which we have found to exist between glycogen and the growth of the fœtus, two pregnant animals were treated with phloridzin for a prolonged period. The treatment had a very marked effect, because the substance probably passed through the placenta and exerted its action directly on the fœtus, and not only mediately through the maternal organism.

0·6 gramme of phloridzin was injected daily into the mother animals from the 8th day of gestation, the time when, according to Chipman, glycogen

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* Burlando, 'Archiv. Ital. di Ginec.,' 1906, p. 246.

Table VI.—Effect of Variation in Diet.

Rabbit A.—Killed 22nd day of gestation. Number of fœtuses, 7.
Diet, carrots and cabbages during pregnancy.

	Maternal liver.	Maternal placenta.			Fœtal placenta.	
	Glycogen percentage.	Average weight per placenta in grammes.	Average weight of "distal" glycogen per placenta.	Glycogen percentage.	Average weight per placenta in grammes.	Average weight of "proximal" glycogen per placenta.
Rabbit A......	6·30	1·56	0·0739	4·60	1·6	0·0045
Control	—	1·73	0·0931	5·40	2·30	0·0082

	Fœtal liver.			Rest of fœtal body.		
	Average weight per fœtus in grammes.	Average weight of glycogen per fœtus.	Glycogen percentage.	Average weight per fœtus in grammes.	Average weight of glycogen per fœtus.	Glycogen percentage.
Rabbit A......	1·0	0·0014	0·14	3·10	0·0087	0·28
Control	0·68	0·0006	0·09	3·45	0·0097	0·28

Rabbit B.—Killed 23rd day of gestation. Number of fœtuses, 6.
Diet, carrots and cabbages for 24 hours before death.

	Maternal liver.	Maternal placenta.			Fœtal placenta.	
	Glycogen percentage.	Average weight per placenta in grammes.	Average weight of "distal" glycogen per placenta.	Glycogen percentage.	Average weight per placenta in grammes.	Average weight of "proximal" glycogen per placenta.
Rabbit B......	7·41	1·67	0·0695	4·16	2·10	0·0059
Control	—	1·63	0·0731	4·50	2·38	0·0062

	Fœtal liver.			Rest of fœtal body.		
	Average weight per fœtus in grammes.	Average weight of glycogen per fœtus.	Glycogen percentage.	Average weight per fœtus in grammes.	Average weight of glycogen per fœtus.	Glycogen percentage.
Rabbit B......	0·68	0·0012	0·17	6·80	0·0184	0·27
Control	0·70	0·0013	0·19	6·50	0·0190	0·29

Table VI—*continued.*

Rabbit C.—Killed 23rd day of gestation. Number of fœtuses, 5.
Diet, carrots and cabbages during pregnancy.

	Maternal liver.	Maternal placenta.			Fœtal placenta.	
	Glycogen percentage.	Average weight per placenta in grammes.	Average weight of "distal" glycogen per placenta.	Glycogen percentage.	Average weight per placenta in grammes.	Average weight of "proximal" glycogen per placenta.
Rabbit C......	5·72	1·52	0·0644	4·24	2·40	0·0072
Control	—	1·63	0·0731	4·50	2·38	0·0062

	Fœtal liver.			Rest of fœtal body.		
	Average weight per fœtus in grammes.	Average weight of glycogen per fœtus.	Glycogen percentage.	Average weight per fœtus in grammes.	Average weight of glycogen per fœtus.	Glycogen percentage.
Rabbit C......	0·76	0·0016	0·21	6·20	0·0143	0·23
Control	0·70	0·0013	0·19	6·50	0·0190	0·29

Rabbit D.—Killed 27th day of gestation. Number of fœtuses, 6.
Diet, carrots and cabbages during pregnancy.

	Maternal liver.	Maternal placenta.			Fœtal placenta.	
	Glycogen percentage.	Average weight per placenta in grammes.	Average weight of "distal" glycogen per placenta.	Glycogen percentage.	Average weight per placenta in grammes.	Average weight of "proximal" glycogen per placenta.
Rabbit D......	4·12	1·18	0·0316	2·68	3·03	0·0015
Control	5·32	1·23	0·0316	2·56	3·93	0·0016

	Fœtal liver.			Rest of fœtal body.		
	Average weight per fœtus in grammes.	Average weight of glycogen per fœtus.	Glycogen percentage.	Average weight per fœtus in grammes.	Average weight of glycogen per fœtus.	Glycogen percentage.
Rabbit D......	2·57	0·0534	2·08	23·7	0·0758	0·32
Control	2·67	0·0392	1·47	30·17	0·1026	0·34

first appears in the maternal placenta. In the first experiment the rabbit (Rabbit E) was killed when 21 days pregnant, and in the second (Rabbit F) when 27 days pregnant. The maternal liver, the placenta, and the fœtal liver and fœtal bodies were analysed.

The results, which are given in Table VII along with the control from the normal series, show very clearly that the placenta does not give up its store of glycogen readily to the maternal organism when the hepatic reserve runs low. The placenta guards its glycogen deposit so much more jealously than the liver that it appears as if the cells of the placenta had a greater affinity for glycogen than those of the liver.

The weight of the placenta is not very much affected in the case of the animal killed on the 21st day of gestation. The differences which are found between the maternal and fœtal parts respectively of the phloridzin animal and the control animal compensate each other, and may perhaps be due to the crudity of the mechanical separation. If the weight of the fœtal and maternal parts are added together, there is practically no difference, the figures being 3·2 for Rabbit E and 3·24 for the control animal.

The placentæ of the animal killed on the 27th day of gestation, which show a distinct diminution in their glycogen store, have been affected also in their growth. Although the weight of the maternal part is slightly higher, the weight of the fœtal part is so low that, even if the total weight of the placenta is taken, a marked diminution is evident. The placenta of Rabbit F weighs 3·2 grammes, that of the control animal 4·13 grammes.

The glycogen of the fœtal livers was estimated only in the second animal, as the first animal was killed at a stage when only traces of glycogen were likely to be present in the fœtal liver. In the animal killed at the 27th day of gestation the weight and glycogen percentage were distinctly lower than in the control animal from the normal series. But the fœtal liver still contained a much higher percentage of glycogen than the maternal liver (0·11 per cent.).

The glycogen of the rest of the fœtal bodies was found to be reduced. At the same time the fœtal weight was decreased in both cases.

The animal killed on the 21st day of pregnancy shows a diminution of the glycogen store of the fœtus, while the placenta has yet retained its full share. A much more profound effect, however, has been produced in the animal killed on the 27th day. Here both the deposit in the placenta and that in the fœtus have been markedly diminished. Associated with these changes there is in both animals a retardation of growth, which again is more pronounced in the animal where the loss of glycogen has been greatest. It should be noted, too, that the retardation of growth is confined to the fœtal

Table VII.

Rabbit E.—Killed 21st day of gestation. Number of fœtuses, 6.

	Maternal liver.	Maternal placenta.			Fœtal placenta.	
	Glycogen percentage.	Average weight per placenta in grammes.	Average weight of "distal" glycogen per placenta.	Glycogen percentage.	Average weight per placenta in grammes.	Average weight of "proximal" glycogen per placenta.
Rabbit E......	—	1·80	0·0850	4·77	1·40	0·0118
Control	—	1·34	0·0746	5·57	1·90	0·0110

	Fœtal liver.			Rest of fœtal body.		
	Average weight per fœtus in grammes.	Average weight of glycogen per fœtus.	Glycogen percentage.	Average weight per fœtus in grammes.	Average weight of glycogen per fœtus.	Glycogen percentage.
Rabbit E......	Not analysed			2·58	0·0045	0·17
Control				2·74	0·0077	0·28

Rabbit F.—Killed 27th day of gestation. Number of fœtuses, 8.

	Maternal liver.	Maternal placenta.			Fœtal placenta.	
	Glycogen percentage.	Average weight per placenta in grammes.	Average weight of "distal" glycogen per placenta.	Glycogen percentage.	Average weight per placenta in grammes.	Average weight of "proximal" glycogen per placenta.
Rabbit F......	0·11	1·50	0·0283	1·89	2·69	0·0003
Control	5·32	1·23	0·0316	2·56	3·93	0·0016

	Fœtal liver.			Rest of fœtal body.		
	Average weight per fœtus in grammes.	Average weight of glycogen per fœtus.	Glycogen percentage.	Average weight per fœtus in grammes.	Average weight of glycogen per fœtus.	Glycogen percentage.
Rabbit F......	1·81	0·0109	0·60	19·25	0·0538	0·28
Control	2·67	0·0392	1·47	30·17	0·1026	0·34

tissues. In the case of Rabbit F, the maternal part of the placenta is not diminished in weight, although its glycogen store has been greatly reduced, while the fœtal part of the placenta, which normally is free from glycogen, is greatly reduced in weight.

The results obtained by treatment with phloridzin confirm the conclusions which we have drawn from our observations on spontaneous cases of retarded growth. They show that the growth of the fœtus is dependent upon glycogen in such a way that the glycogen is not actually present in the growing cell, but that the fœtus draws upon a deposit which is laid down at first in the maternal part of the placenta and is transferred in the last week of pregnancy to the liver of the fœtus.

F. *The Glycogen-splitting Ferment of the Placenta.*

Glycerine extracts, both of the maternal and of the fœtal part of the rabbit's placenta, were found to have a powerful hydrolytic action on glycogen. This action was destroyed by heating the extracts. Extracts of sheep's placentæ showed, even on qualitative examination, a very much less marked glycogen-splitting power.

The activity of the ferment was measured quantitatively by determining the amount of sugar split off after incubating a mixture of measured quantities of the glycerine extract and of a solution of glycogen, purified by repeated precipitation with alcohol. Toluol was added. After 24 hours the glycogen was precipitated by alcohol and the amount of sugar estimated in the filtrate by Allihn's method. In order to account for the sugar of the blood present in the glycerine extracts, control experiments were made with the heated extract. In these controls only very small and almost constant quantities of cuprous oxides were found, the figures ranging from 0·001—0·004 gramme. These figures were subtracted from the results obtained in the digests, and in this way a measure of the activity of the ferment was obtained.

The results obtained for the placenta of the rabbit are given in the following table:—

Glycogen-splitting Ferment in Placenta of Rabbit.

Day of gestation.	Maternal placenta. Weight of Cu_2O. (Difference.)	Fœtal placenta. Weight of Cu_2O. (Difference.)
	gramme.	gramme.
17	0·0218	0·0328
21	0·0040	0·1463
24	0·0424	0·0476
30	0·0196	0·0237

Extracts of the placenta of the sheep and the cow were examined in the same way, and gave the following result:—

Glycogen-splitting Ferment in Placentæ of Sheep and Cow.

Species.	Maternal placenta. Weight of Cu_2O. (Difference.)	Fœtal placenta. Weight of Cu_2O. (Difference.)
	gramme.	gramme.
Sheep	0·0084	0·0052
,,	0·0068	0·0080
Cow	negligible	negligible
,,	0·0060	0·0040

The results reveal a marked difference between the placenta of the rabbit and that of Ruminants, the last-named animals yielding a much weaker ferment than the rabbit. It is a very interesting fact that this difference is reflected also in the glycogen content of the placentæ of these animals.

The maternal and fœtal cotyledons of the placenta of the sheep were examined for glycogen in several cases. The material was obtained from the slaughter-house and extracted within half an hour after death. By extraction with hot acidulated water we failed to find any glycogen. Experiments with Pflüger's method of extraction with strong alkali, however, showed that the fœtal cotyledons of the sheep's placenta contain glycogen in traces to the amount of about 0·01 per cent.

The glycogenic changes in the sheep's placenta must therefore be very insignificant, and correspondingly we find a weak glycogen-splitting ferment in this case. In the rabbit, on the other hand, where large quantities of glycogen are dealt with by the placenta in a short time, we find a powerful ferment present in the placenta. The glycogen of the maternal placenta of the rabbit, which, as we have seen, is made use of by the embryo, must necessarily pass through the fœtal part of the placenta. This part contains such small traces of glycogen, if any at all, that they cannot be detected by microchemical methods. We must assume, then, that the plasmodium absorbs glycogen not as such, but in an altered form. At the same time we find that the fœtal part of the placenta secretes a strong glycogen-splitting ferment, more powerful even than that of the maternal part.

In view of these facts, it is difficult to avoid the conclusion that in the placenta the ferment is active during life. If we assume that it is found only after death, we would meet with the further difficulty, that a tissue—the fœtal part of the rabbit's placenta—which contains no glycogen during life should suddenly develop a glycogen-splitting enzyme after death.

The objection that the glycogen-splitting power of the placenta is due to the presence of blood, which is known to have a diastatic action, is negatived by our results. The extracts of the placenta contained as much blood in Ruminants as in rabbits, yet the diastatic power of the placenta of the former was almost negligible compared with that of the latter.

We wish to point out specially that the glycogen-splitting enzyme is an enzyme secreted by the cells. It is the only enzyme which we have been able to demonstrate in glycerine extracts of the placenta, although a large number of experiments were made to test for the presence of tryptic, peptic, ereptic, and lipolytic enzymes in these extracts. All these experiments gave negative results. That such ferments are present within the cells of the placenta as intracellular enzymes, and that their presence can be demonstrated by using more severe methods than extraction with glycerine, we do not doubt. But such ferments, with the sphere of their activity limited to the cell itself, are present in the cells of many if not of all organs, so that their presence in the placenta would not throw much light on the function of this organ.

On the Fate and Function in the Fœtal Organism of Glycogen absorbed from the Placenta.

Whatever the source of the placental glycogen may be, its presence cannot be due to the accident of the anatomical site of the rabbit's placenta, which, although identical in position with that of the cow and sheep, is distinguished by its power of forming and storing glycogen. We cannot but recognise a definite function of the cells of the rabbit's placenta of depositing a store of carbohydrate for the fœtus.

There can be little doubt that the placental glycogen is absorbed by the plasmodium. It is situated, as we have seen, in decidual cells which are intimately related to the ectodermic tubules, and are, indeed, in Chipman's words, "swallowed" by them, so that the glycogen contained in them must also be absorbed into the fœtal structures.

The quantitative relationship which we have proved to exist between the decrease in the placental glycogen and the increase in the fœtal glycogen affords strong direct evidence in support of the view that the placental glycogen forms a store for the fœtus.

A specific function appears to arise in the fœtal liver in the last week of gestation. If the hepatic store of glycogen were due to the favourable anatomical position of the fœtal liver, we should expect to find the glycogen contents of the liver to be above that of the rest of the body during the whole period of gestation. We have seen, however, that up to the 24th day the function of storing glycogen for the fœtus has not yet been acquired by the liver and is, in the meantime, fulfilled by the placenta.*

The glycogen metabolism of the placenta and the fœtus differs in some essential points from that of the adult organism. In the latter case the glycogen deposited in the tissues represents a store dependent upon external conditions and showing irregular fluctuations. The placenta and the fœtus, on the contrary, are characterised by the absence of individual variations in the amount of glycogen they contain at a given period of gestation. The glycogen metabolism of these organs shows a regular succession of changes which proceed almost regardless of external conditions, and which are independent to a great extent even of the glycogen metabolism of the mother.

These facts point to the conclusion that in the development of the fœtus glycogen fulfils a definite function and is not merely an accessory source of nutritive energy as it is in the adult organism. Part of the glycogen which is absorbed from the placenta may be accounted for by the intense carbohydrate metabolism, which, according to Bohr's† experiments on the gaseous metabolism, proceeds in the fœtal rabbit. Bohr's results are not in agreement with those of Cohnheim and Zuntz.‡ But since the latter observers worked with sheep, which, in view of our observations, may have a different metabolism of pregnancy, and since Bohr's figures show that his method entailed very little interference with the fœtus, his observations may be taken as representing the normal condition in rabbits. The glycogen which is thus catabolised furnishes thereby the energy necessary for the formation of new tissues, the "Entwicklungsarbeit" of Tangl.§

The question arises whether glycogen performs also anabolic functions in the development of the fœtus.

The absence of glycogen from some of the growing fœtal tissues, and the fact that the tissues where it is present do not contain even as much as the adult ones, leave little doubt that a definite formative power cannot be attributed to glycogen as such. On the other hand, the scarcity of glycogen in embryonic tissues does not necessarily justify the conclusion that glycogen does not take part in the building up of the tissues. It is well known that embryonic tissues are rich in mucin, which contains a large amount of a carbohydrate group in its molecule. Although glycogen as such has no formative power, it may yield one of the "Bausteine" for the building up of the main protein body of fœtal tissues. In this connection it is interesting to consider the conditions in the hen's egg, which must necessarily contain in itself the material of which the embryo is built up. In the egg, carbohydrate as such, in the form of either glycogen or glucose, is almost absent. At the same time all the protein substances of the white of egg are distinguished by containing a large amount of glucosamine in their molecule. In the egg, therefore, the carbohydrate group has already entered into the protein molecule, and correspondingly there is a scarcity of free carbohydrate.

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* In order to account for the behaviour of the hepatic glycogen during development on purely anatomical grounds it would be necessary to assume that at the beginning of the last week of pregnancy the vascular supply of the liver undergoes a complete change, so that after that date the fœtal liver is freely supplied with blood from the placenta, while before that date very little blood from the placenta reaches the liver. We are not aware, however, that such a relation between vascular changes and the storing of glycogen can be established.

† Bohr, 'Skandinavisches Archiv für Physiologie,' vol. 10, 1900, p. 413; vol. 15, 1904, p. 23.

‡ Cohnheim and Zuntz, 'Pflüger's Archiv,' vol. 34, 1884, p. 173.

§ Tangl, 'Pflüger's Archiv,' vol. 93, 1903, p. 327.

Summary.

In an age-series of pregnant rabbits from the 14th day of gestation to the end of pregnancy, quantitative estimations were made of the glycogen present in the placenta, in the liver of the fœtus, and in the rest of the fœtal body. The mechanical separation of the placenta into the maternal and the fœtal part made it possible to determine the amount of glycogen in the peninsulæ of the maternal placenta, which are most intimately associated with the fœtal placenta, apart from the glycogen in the rest of the maternal placenta.

A marked feature of the results is the absence of individual variations in the glycogen contents of the various organs. Instead, there is an orderly sequence of changes, which allows of a graphical expression.

Glycerine extracts of both the maternal and the fœtal parts of the rabbit's placenta contain an active glycogen-splitting enzyme. No evidence was obtained for the presence in these extracts of a proteolytic or lipolytic enzyme.

Comparative observations on the placenta of the sheep show that the paucity of glycogen in the placenta of this animal is associated with the presence of a very weak glycogen-splitting enzyme in the glycerine extracts from this organ. The glycogen store of the rabbit's placenta is not increased by feeding the mother animals on a diet rich in carbohydrates. Nor has such a diet any effect in increasing the hepatic glycogen reserve of the fœtus either in the earlier or in the later stages of pregnancy. On the other hand, the placenta does not give up readily its store of glycogen to the mother organism when the hepatic reserve of the mother is exhausted by conditions such as injections of phloridzin. Neither during gestation nor at birth does the percentage of glycogen in the fœtal organs reach the average amount found in adult tissues.

There is a distinct parallelism between the growth of the fœtus and the percentage amount of glycogen which it contains. In two cases where growth had been spontaneously arrested, the percentage amount of glycogen in the fœtal organs was diminished out of proportion to the diminution in weight. A similar condition could be reproduced experimentally by repeated injections of phloridzin.

The interpretation of these results has led to conclusions which may be summarised as follows:—

The placenta of the rabbit has the function of depositing glycogen as a store of carbohydrates for the needs of the fœtus. The glycogen is absorbed from the maternal placenta in the form of a simpler carbohydrate. This transformation takes place in the placenta and is brought about by the action of an enzyme secreted by the placenta.

In the earlier stages of intra-uterine life the fœtal liver is devoid of the power of storing glycogen, which it does not acquire until the last week of gestation. Before that date the placenta vicariously fulfils the hepatic function as far as glycogen is concerned. The glycogen metabolism of the placenta and the fœtus is independent of that of the mother, and appears to be governed by conditions different in many respects from those which regulate the glycogen metabolism in the adult animal.

There is a distinct relation between the glycogen metabolism and the growth of the fœtus. Since the growing tissues of the fœtus are not distinguished by an abundance of glycogen, a definite formative power cannot be attributed to glycogen *quâ* glycogen. The function of glycogen in the development of the fœtal rabbit is probably to furnish material, on the one hand, for the intense carbohydrate metabolism that proceeds in the fœtus; and, on the other hand, for the building up of the protoplasm of fœtal tissues.

The expenses of this investigation were defrayed by grants from the Moray Fund of the University of Edinburgh.

On the Maturation of the Ovum in the Guinea-pig.

By J. E. SALVIN MOORE, A.R.C.S., F.L.S., Professor of Experimental and Pathological Cytology, Liverpool, and Miss F. TOZER, B.Sc. London.

(From the Cytological Department of the University of Liverpool.)

(Communicated by J. Bretland Farmer, F.R.S. Received January 14,—Read February 27, 1908.)

[PLATES 5—7.]

The following observations on the maturation of the ovum in the Guinea-pig are related to a more extended investigation. Certain results which we have already obtained while investigating the development of the eggs of the Guinea-pig are, however, of interest, and we think it desirable to give a brief account of them.* In the earlier stages of the development of the eggs within the ovary they appear as cells which present within their nuclei the characteristic synaptic contraction of the nuclear thread-work. Occasionally, during such stages, the centrosomes are clearly visible within a definite archoplasm. As the eggs increase in size the centrosomes appear to migrate from the interior of the archoplasm into the cytoplasm this migration corresponding to the migration of the centrosomes in similar stages in the development of spermatozoa. At a later period in the development, both archoplasm and centrosomes in the eggs disappear altogether.

The development of the egg is nearly complete within the ovary. That is to say, the two maturation spindles corresponding to the maturation spindles in the production of the spermatozoa are found while the eggs are enclosed in the follicle and are still retained in the ovary.

When the eggs have reached the large size represented in Plate 5, 1, the final stages in the evolution of a coarse spirem, corresponding to the coarse spirem of the spermatogenesis, are reached, and in the succeeding phase there occurs the usual development of gemini within the nucleus. In a large number of the eggs the gemini appear to result from the pairing of the number of chromosomes usually found in somatic cells; but it is very important to note that in many of the eggs with which we have had to deal the number of the aggregates of chromatin is largely in excess of what it ought to be, supposing these bodies normally occur in half the number of the somatic chromosomes. When the eggs have reached the above condition, the first polar body spindle is formed in the manner represented in fig. 2. There are no centrosomes, and several of the characteristic forms of the gemini correspond to the forms seen in the similar stages of spermatogenesis. The division of the gemini takes place in the same manner as that occurring during spermatogenesis and the diaster of such a division is represented in fig. 3. When the first polar body has been definitely extruded, as in fig. 4, the nucleus which remains in the egg immediately proceeds towards another division; the chromosomes appearing on this spindle (the second maturation spindle) being in the form of rods or diads, such as those represented in fig. 6. Here, again, as in the case of the first polar body spindle, we have found in some cases that the number of chromosomes appearing is greater than half the somatic number. In the second polar body spindle, as in the first, there are no centrosomes. At the time the second polar body is being produced, it is often possible to observe a division of the nucleus of the first polar body also taking place, and the final result is the production of four nuclei, one remaining in the egg, two derived from the first polar body, and one belonging to the second polar body. We are at present inclined to interpret the high number of gemini and chromosomes appearing during the first and second polar body divisions as possibly due to the fact that in the first polar body division the synaptic chromosomes have not all united in pairs to form gemini before the division ensues; whilst during the second polar body division the number perhaps appears to be high, owing to the fact that when the rods and diads are produced they often become prematurely divided, so that, in the act of counting, halves may be reckoned as whole individual chromosomes.

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* A detailed account of the early development of the egg in mammals is given by Winiwarter ("Recherches sur l'Ovogenèse et l'Organogenèse de l'Ovaire des Mammifères (Lapin et Homme)," par le Dr. Hans von Winiwarter, 'Archives de Biologie,' vol. 17, 1900). An account of the fertilisation and segmentation of the eggs of the Mouse is contained in a paper by Sobotta in the 'Arch. f. Mikr. Anat.,' vol. 45, 1895.

In a certain number of eggs a very interesting process can be observed resulting in the parthenogenetic segmentation of the eggs, and the process may extend to the production of 12 or 15 irregular blastomeres; it then comes to an end, the eggs apparently degenerating. The spindles found in these dividing eggs are typically somatic in appearance.

This process is probably the same as that described and figured by J. Janosik in his account of the "Atrophy of the Follicles of the Guinea-pig,"* but it is necessary to draw attention to the fact that Janosik may possibly have missed the point upon which we lay stress. He has failed to separate the process resulting in the production of polar bodies from that ending in irregular segmentation and degeneration of the egg cell, and he apparently considers the stage showing a polar body and the spindle, for the

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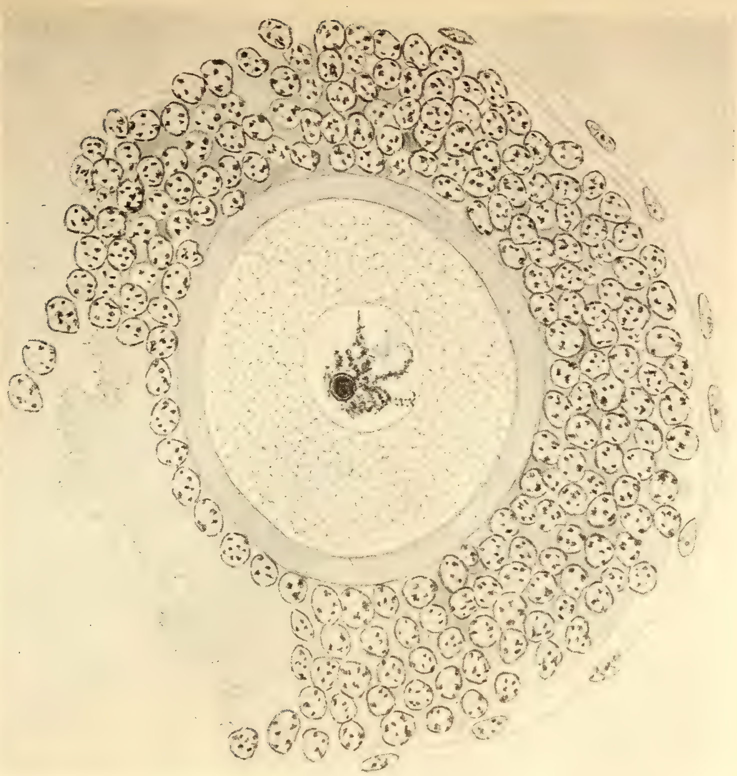
* "Die Atrophie der Follikel und ein seltsames Verhalten der Eizelle," 'Arch. f. Mikr. Anat.' vol. 48, 1897.



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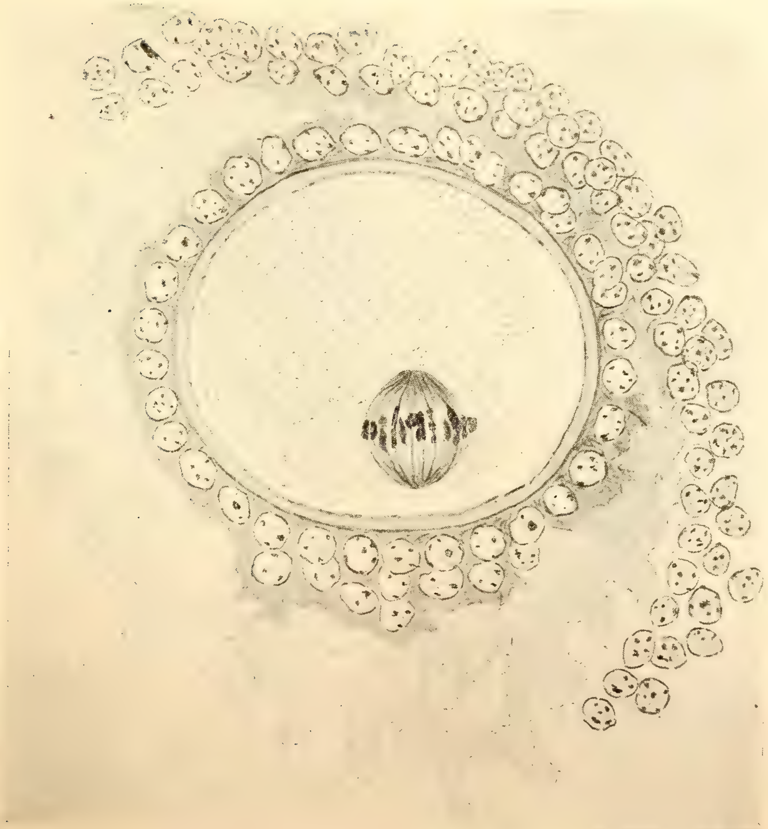

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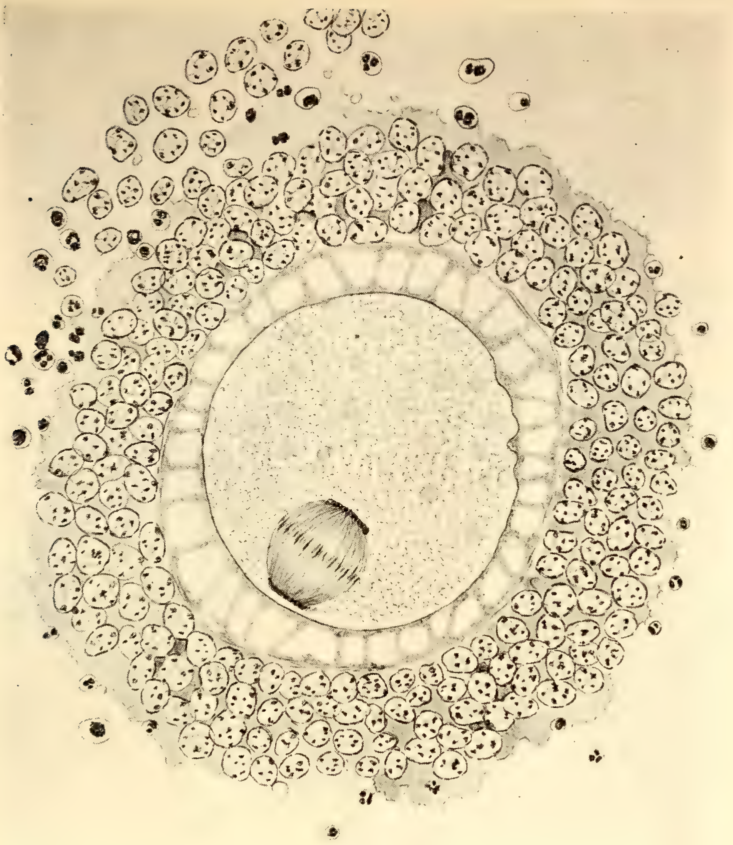

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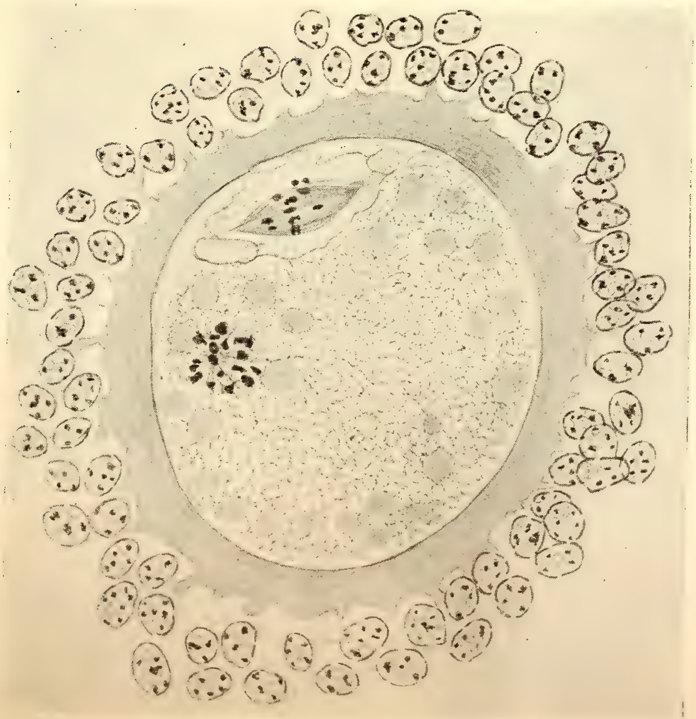

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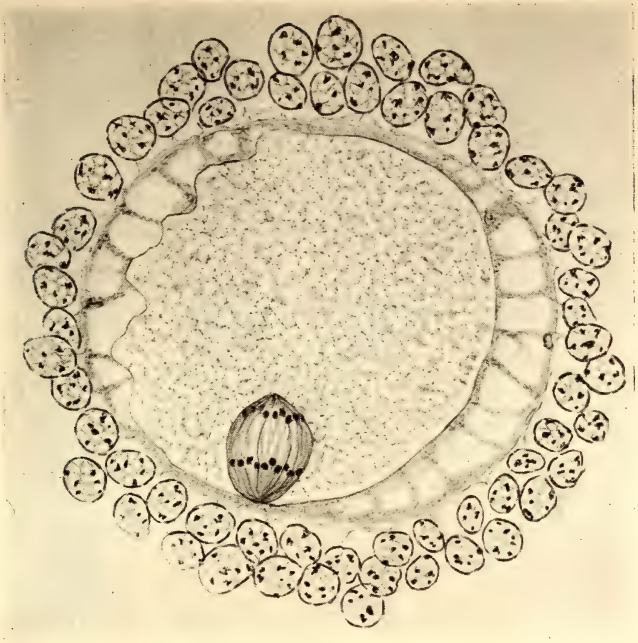

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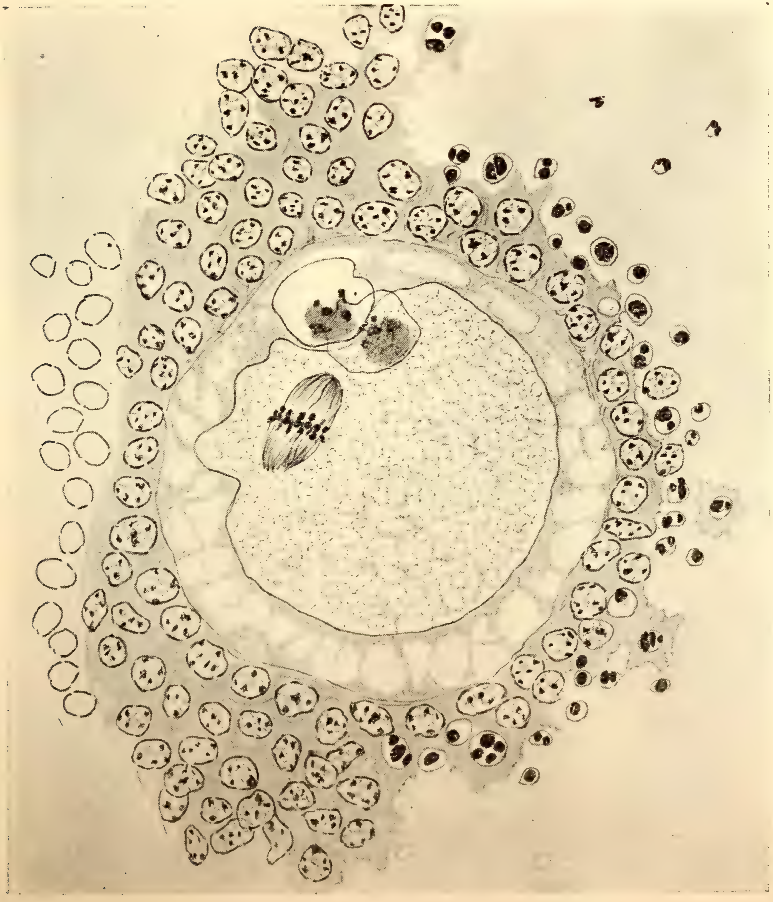

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production of the second polar body (which he figures), to be merely the first segmentation of an egg which has entered upon a process of degeneration and the follicle of which is about to atrophy.

DESCRIPTION OF PLATES.

Plate 5.

Fig. 1.—Ovum of Guinea-pig. The nucleus in a late stage of synapsis.

Fig. 2.—Ovum of Guinea-pig, showing the spindle (first polar body or heterotype spindle).

Plate 6.

Fig. 3.—Ovum of Guinea-pig. The nucleus is in the diaster of the meiotic division (*i.e.*, the formation of the first polar body). The intermediate bodies of Flemming are also seen on the fibres at the equator of the spindle.

Fig. 4.—Ovum of Guinea-pig, showing the post-meiotic spindle (polar view) and the first polar body. The latter is itself in division.

Plate 7.

Fig. 5.—Ovum of the Guinea-pig, showing diaster of meiotic spindle.

Fig. 6.—Ovum of Guinea-pig, showing the post-meiotic spindle, or second polar body spindle. The first polar body has divided and its two daughter elements are together on the upper margin of the cell.

The Life-history of Trypanosoma equiperdum.

By J. E. Salvin Moore, Professor of Experimental and Pathological Cytology, University of Liverpool, and Anton Breinl, Director of the Runcorn Research Laboratories, Liverpool School of Tropical Medicine.

(Communicated by Sir Rubert Boyce, F.R.S. Received March 9,—Read March 12, 1908.)

[Plates 8 and 9.]

In May, 1907, we* showed that the study of the parasite of sleeping sickness (*Trypanosoma gambiense,* Dutton), as it appears in the blood of rats artificially infected with the disease, revealed a cyclical metamorphosis, and that this cyclical metamorphosis corresponded closely to the alternating phases of presence and absence of trypanosomes in the blood. At the same time it was found that the cyclical metamorphosis in the parasites corresponded less closely, but still unmistakably corresponded, with the successive alternations of condition that characterise the clinical aspects of the malady in the host. The features of the life-cycle of the parasites of sleeping sickness as they appear in the blood during infection in rats are remarkable, and may be briefly repeated for reference. From the time of inoculation the parasites multiply in the blood through amitotic division of the nucleus,† and longi-

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* Note on "The Life-history of the Parasite of Sleeping Sickness," 'Lancet,' p. 1219, May 4, 1907; "The Cytology of the Trypanosomes," Part I, 'Ann. Trop. Med. and Parasitology,' vol. 1, No. 3.

What is called the nucleus of those trypanosomes with which we are acquainted, when fixed in Flemming's fluid, or by any other appropriate method, does not appear to show any trace of chromosomes. It consists of a central sphere, intra-nuclear centrosome (Salvin Moore and Breinl, *loc. cit.*) enclosed by a mass of substance, which may be made to stain in a different manner from the sphere. When the nucleus divides, the interior sphere first elongates, then assumes a dumb-bell shape, and finally breaks into two spheres, the outer substance collecting these new centres (intra-nuclear centrosomes), so as to form two smaller nuclei with the same structure and appearance as the first. The reasons for regarding this structure as a nucleus, *i.e.*, as equivalent in a morphological sense to the nuclei of other protozoa, and protophyte bodies, and to the nuclei of the metazoa and metaphites, are simply these: The structure in question bears a superficial resemblance to the nuclei with which biologists are familiar. It divides in the amitotic fashion, *i.e.*, as if it were a viscous drop, and owing to the existence of the intra-nuclear centrosome, and to the manner in which this body appears initiate the fission, the whole structure bears a close and striking resemblance to the undoubted nuclei of some unicellular organisms, such as Euglena. There is, however, this difference: the nuclei to which that of the trypanosomes bears the closest resemblance, such as those of the Euglenæ, have been found to possess chromosomes. Chromosomes have been described as appearing during the divisions of the nuclei of tudinal fission of the trypanosomes, until a vast number of trypanosomes are produced and the infection of the blood reaches a first maximum. (See chart, p. 290.)

After such a period has been reached, the number of parasites in the blood falls until it may be impossible to detect their presence. But subsequently parasites reappear, and a second maximum is reached, and so on. The alternations of these maxima and minima in rats during an infection with *T. gambiense* are illustrated in the chart given on p. 290. In the case of man,

trypanosomes by Schaudinn, Prowazek, Minchin, and others, but we have been uniformly unable to confirm these observations, and have reached a diametrically opposite conclusion, namely, that in the case of *Trypanosoma gambiense*, *T. equinum*, *T. lewisi*, *T. brucei*, and *T. equiperdum*, chromosomes are not present, and do not exist, at any rate during those forms of division which take place in the blood of a mammal infected with these trypanosomes.

[*Footnote added April* 13, 1908.]

Minchin, in the 'Quart. Journ. Micro. Sci.,' vol. 32, Part II, describes the structure we term extra-nuclear centrosome as the kineto-nucleus, and a different structure, a small swelling or bead, at the end of the stainable portion of the flagellum, as the blepharoplast or centrosome. The reasons for regarding the body we term the extra-nuclear centrosome as a centrosome are as follows :—

The extra-nuclear centrosome appears to be derived from the intra-nuclear centrosome, and the intra-nuclear centrosome (karyosome nucleolus) appears to be a structure which is closely similar in its appearance and behaviour to the undoubted intra-nuclear centrosomic, or blepharoplastic, bodies within the nuclei of Euglena, and many protozoa. This conception is strengthened by the fact, originally observed by one of the present authors in 1894 (Moore, 'Internat. Monatschr. f. Anat. Physiol.,' vol. 11), that the true metazoan centrosome is regularly incorporated within the flagellated male gametes of these organisms. In such gametes the centrosomes become more or less definitely related to the flagella, just as the extra-nuclear centrosome is related to the flagella of the trypanosomes. In many metazoan gametes (reptiles) the flagellum abuts directly upon the centrosome. In others (some mammals) this is not so, the flagellum ending in a small bead corresponding to the blepharoplast of Minchin. In such cases the true centrosomes remain quite detached, as in some trypanosomes, for example in *T. lewisi*. The bead, when it exists on the base of the metazoan flagellum, is not a centrosome, but simply an enlargement of the proximal end of the flagellum. For this reason we do not regard the blepharoplast of Minchin as equivalent to the blepharoplastic, or centrosomic, structures of other cells, but we regard the bead in question as possibly equivalent to the swelling at the end of the flagellum found among many metazoan cells and gametes. Similarly, we regard the name kineto-nucleus, when applied to what we call the extra-nuclear centrosome, as entirely inappropriate. In the first place, in so far as this structure can be homologised with any structure known in other cells, it appears, as we have said, to have the same relationships as the centrosome. In the second place, it does not appear to have any attributes, except the capacity to divide (a capacity which, of course, is shared by all centrosomes), in common with what is understood as a nucleus. Minchin draws attention to the large size of the extra-nuclear centrosome (kineto-nucleus, nucleolus blepharoplast) in some trypanosomes, as indicating that this structure is not of the nature of a centrosome ; but we are unable to see that the dimensions of this body affect the matter in any way, for the undoubted centrosomic or blepharoplastic structures of many male plant gametes are similarly large, if not larger.

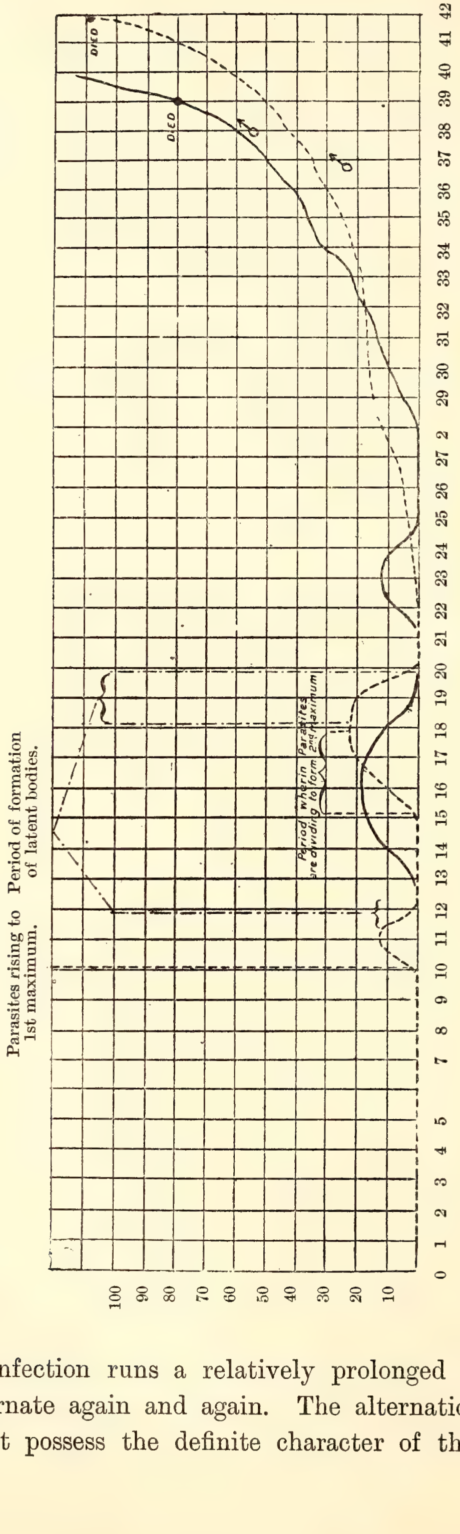

Chart of Two Male Rats inoculated with *Trypanosoma gambiense*.

The horizontal figures represent the days after inoculation: the vertical figures the numbers of parasites in a microscopic field of blood, the curve representing the variation in this during the course of the infection. The two different curves represent two different infections.

where the infection runs a relatively prolonged course, the maxima and minima alternate again and again. The alternation is somewhat irregular, and does not possess the definite character of the analogous periods in a typical malarial infection. In the case of *T. gambiense* it has been found* that amitotic division of the parasites proceeds up to the first maximum, but at this period other changes are also apparent in the parasites of the blood (see Diagram I, *a*, *b*, *c*.) At this period numbers of parasites may be found in which a thick band is seen to be growing out from the extra-nuclear centrosomes (blepharoplast) which lies about the base of the flagellum (Diagram I, *b*). This band extends down the interior of the body towards the nucleus. After a time the band enters into connection with the nucleus, and then breaks up and disappears.† At or near the maxima there is thus an interaction between the extra-nuclear centrosome and the nucleus. When the maximum has

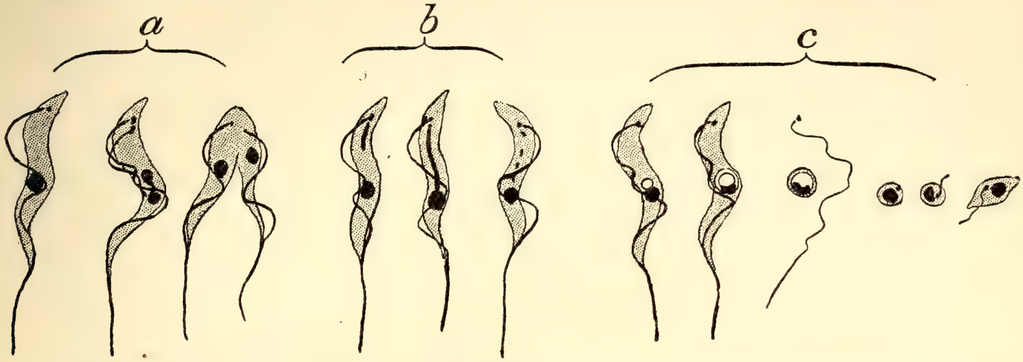

Diagram I.—Showing the cyclical metamorphosis occurring in *Trypanosoma gambiense* in the blood from an infected rat. *a*. The longitudinal fission of the parasites. *b*. The interaction between the extra-nuclear centrosome and the nucleus. *c*. The formation of the latent bodies and the early stages of their redevelopment into trypanosomes.

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* Salvin Moore and Breinl, *loc. cit.*

† Referring to our communication, 'Annals of Tropical Medicine and Parasitology,' *loc. cit.*, Swellengrebel ('Compt. Rend. Soc. Biol.,' vol. 64, 1908, No. 2) appears to regard the formation of the stainable band as in some manner due to a form of degeneration. We are, however, entirely unable to agree to this conception for the following reasons. The formation of the band occurs only at a particular period of the infections, and at other periods it is not induced either by the administration of substances such as atoxyl or by allowing the trypanosomes to die. A clearer demonstration of the erroneous conclusion drawn by Swellengrebel is, however, found in the facts relating to *T. equiperdum;* here a similar process regularly occurs (see this paper) and after it has occurred the trypanosomes again pass through division, this fact demonstrating that the process to which we refer can have nothing to do with degeneration and is, on the contrary, part of a cycle occurring during the ordinary course of development.

Swellengrebel appears to us to be again in error in supposing that the formation of the stainable band is connected with the production of trophic granules. That this is not the case can readily be seen by the use of stains such as that of Breinl or modifications of the iron hematoxylin method, whereby the remains of the band and the band itself stain quite differently to the trophic granules. Moreover, before any stainable band is produced the trophic granules are numerous and must have arisen from some other source.

been reached, the parasites in the blood rapidly diminish in number, and during the period of diminution large numbers of trypanosomes may be encountered in the lungs, the spleen, and the bone-marrow (but at the same time also in the blood), wherein a profound and rapid change is taking place. The nucleus becomes more compact (see Diagram I, *c*). There arises a vesicle in connection with it, and eventually a complex structure (latent body) is produced,* consisting of the nucleus and vesicle, and enclosed by a delicate covering of cytoplasm. The latent body becomes detached from the rest of the protoplasm of the cell, and the whole remaining portions of the trypanosome now rapidly degenerate and disappear, so that in a short time we find nothing but numbers of the complex spherical latent bodies remaining. These eventually become chiefly lodged in the spleen, the bone-marrow, and other organs. The process we have just described, or, at least, parts of it, have undoubtedly been seen in other trypanosomes, but not in Gambiense, by various observers, and they have generally been interpreted as a form of degeneration. That this is not necessarily so seems now, however, to have become clearly demonstrated. In the case of *T. gambiense* we were able to find, during the period when no parasites were present in the blood, that the latent bodies still persisted in the organs, and that many of these gradually grew larger by developing a cytoplasmic investment, a new extra-nuclear centrosome, and afterwards a flagellum, such forms returning eventually to the form of ordinary trypanosomes.

We have thus in the case of the sleeping sickness parasite a cyclical metamorphosis going on in the blood of the infected animal. The parasites pass through divisions until the interaction between the extra-nuclear centrosomes and the nucleus. From this period they proceed to the formation of latent bodies, and subsequently to the development from the latent bodies of ordinary trypanosomes once more.

The interaction between the nuclei and the extra-nuclear centrosome (blepharoplast) may, as we have pointed out,† suggest a novel form of sexual process, and the whole series of changes may indicate that the life-cycle of *T. gambiense* is in reality completed in the body of the rat or man, and not necessarily related to the transference of the parasites to any other form of host. We know, however, that in the form *T. gambiense* the parasites can be transmitted by the fly *Glossina palpalis.*

The observations of Schaudinn apparently indicate that in the case of *Trypanosoma noctuæ* the sexual stage (which is described by him as quite unlike the process to which we have just drawn attention) occurs in the body of a mosquito. This mosquito appears thus to stand in the same relation to the owl *Athene noctua* as *Glossina palpalis* does to man. It is perfectly natural therefore to suppose that whatever cycle we may have found in the blood during infections with *T. gambiense,* the real sexual phase may occur within *Glossina palpalis* or some other fly. From the information which at present exists this line of criticism cannot be answered through observations upon infections with *T. gambiense.* On this account, and pending further investigation in relation to *T. noctuæ,* we have turned our attention to the parasites of the horse disease known as "Dourine," and caused by *T. equiperdum.* Dourine* is not necessarily transmitted through any fly or intermediate host, but by direct contact between animals that have become infected. In this case we have, then, a trypanosome the life-history of which is not necessarily complicated by transference through an intermediate host. Whatever sexual phase there may be in the life-history of this parasite must be passed through in the body of the horse.

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* Salvin Moore and Breinl, *loc. cit.*

† Salvin Moore and Breinl, *loc. cit.*

If *T. equiperdum* be injected into rats, the parasites multiply and kill the animal in about four days after their first appearance in the blood, which occurs about three days after the inoculation.† The disease in rats thus reaches a first maximum, and the animal dies without being able to overcome the invasion even temporarily, as would appear to be the case in infections produced by *T. gambiense* in rats. The method of investigation has been as follows:—From the time the trypanosomes appear in the blood a very large number of slides have been prepared at short intervals up to death, and for a short time afterwards. Owing to the manner in which the disease is transmitted, special attention was paid to the fluids which collect in the various superficial swellings that are produced, for it naturally seemed possible that a phase of the life-history might occur in such positions in relation to the transmission of the parasites. The results of a prolonged investigation of this matter have, however, revealed nothing but the presence of ordinary trypanosomes,‡ and it is thus indicated that the transference takes place by means of the ordinary trypanosome encountered in the blood, possibly through the existence of slight abrasions on the animals that become infected, or more probably through the capacity of the trypanosomes to invade a mucous membrane, even if it is intact.

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* The strain we have used was obtained through the courtesy of Geheimrath Professor Ulenhuth, in Berlin.

† The best general account of dourine is contained in the works of Laveran and Mesnil's 'Trypanosomes et Trypanosomiases,' Paris, 1904.

‡ In rabbits, when few trypanosomes appeared in the blood it is interesting to note that in the fluids from the swollen vagina there existed many more parasites than in the blood.

Preparations of the blood during early stages of infection show the trypanosomes to be increasing in numbers through rapid longitudinal fission, accompanied by amitotic division of the nucleus and the extra-nuclear centrosomes (Plate 8, figs. 1—4). As the disease advances, two series of structural changes in the parasite become apparent; one of these (figs. 6—10) consists in a gradual increase of the nuclear substance towards the side away from the extra-nuclear centrosome, until, after forming a distinct protuberance, the mass separates from the nucleus and passes away towards the free end of the flagellum in the manner represented in figs. 8, 9, and 10.

It is only possible at present to describe the existence of this process. It may be related to the formation, or rather the renewal, of the so-called "trophic granules,"* but we cannot decide this matter at the present time. The other process to which we have referred is of a totally different order. Towards the end of an infection, that is to say, on the third day after the appearance of the parasites in the blood, numbers of trypanosomes are observed, wherein the extra-nuclear centrosomes become conspicuously large (figs. 11, 12), and at the same time there exist others, in which it is seen that the extra-nuclear centrosome is budding off a large mass towards the nucleus. This mass becomes detached, and can be found in many individuals passing away towards the nucleus (figs. 12, 13, 14). There is often a distinct, but faint, suggestion of a protoplasmic thread still connecting the detached body with the extra-nuclear centrosome at the base of the flagellum (figs. 12, 13, 14). While this process is going on the intra-nuclear centrosome moves towards the extra-nuclear centrosomes, as in figs. 13, 14, and in a number of trypanosomes stages may be found in which the detached portion of the extra-nuclear centrosome is seen to pass completely through the body of the trypanosome until it becomes applied to the nucleus, as in figs. 15, 16. Some time later, the detached portion of the extra-nuclear centrosome merges with, and becomes indistinguishable from, the nuclear substance, and the trypanosomes again pass through division, as in figs. 16, 17 (Plate 9). As division sometimes begins again before the detached centrosome is fused with the nucleus, the process can have nothing to do with any sort of degeneration.

The process we have just described in the parasite of dourine is clearly analogous to the production of the stainable band in *T. gambiense*. It differs only in there being a detached mass which passes from the extra-nuclear centrosome to the nucleus instead of a continuous band. As a matter of fact, the difference is really less than this, for in *T. equiperdum* the extra-nuclear centrosome not only seems to be connected with the detached portion, but also several portions may be detached, one after another. (Compare Diagrams I and II, *a*, *b*, *c*.)

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* It is curious to note that Laveran and Mesnil, *loc. cit.*, p. 273, are under the impression that one of the specific characters of *T. equiperdum* is constituted by the absence of protoplasmic granulations; whereas, on the contrary, we have found them in abundance. It is impossible to say upon what this difference of condition depended.

The phase wherein the detachment of a portion of the extra-nuclear centrosome which passes to the nucleus takes place is of very short duration, and at the same time appears to affect the great majority of the parasites present in the blood, just in the same way that an epidemic of conjugation among infusoria will affect at the same time a whole colony. In the specimens from which the figures are given the process was in full swing at 10 A.M., but by 5 P.M. had come to an end.

At the time this process is going forward, and immediately afterwards, still further changes take place, corresponding to the changes which in *T. gambiense* precede the production of the latent bodies. But the latent bodies of dourine are very much larger than those in *T. gambiense*, and consequently their formation is proportionately less difficult to elucidate.

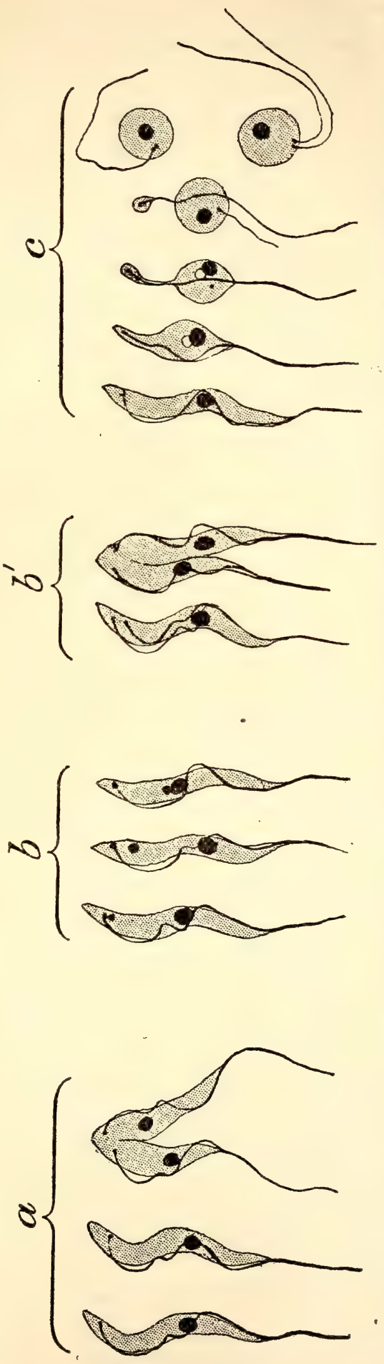


DIAGRAM II.—Showing the cyclical metamorphosis occurring in *Trypanosoma equiperdum* in the blood of an infected rat. *a*. The longitudinal fission of the parasites. *b*. The interaction between the extra-nuclear centrosome and the nucleus. *b′*. The longitudinal fission which occurs after the interaction between the extra-nuclear centrosome and the nucleus. *c*. The formation of the latent bodies and the growth of their flagellæ.

When the process of translocation of a portion of the extra-nuclear centrosome has come to an end, and the parasites have again passed through several longitudinal fissions, a certain number of them are seen to become shorter, and when subject to the action of Breinl's stain are relatively blue, in contrast to the purple coloration of the remaining parasites (figs. 19, 20, 21, 22). In such altered forms it is seen that the extra-nuclear centrosomes become related to a protoplasmic elongation, produced perhaps by the rounding up of the protoplasm of the animal's body (figs. 20—22). This elongation becomes finally club-shaped, and the extra-nuclear centrosome, together with the flagellum, becomes detached from the rest of the cell, which is now more or less round (figs. 21—23). In many cases quite a considerable portion of protoplasm is detached along with the extra-nuclear centrosome, and the detached structure may present, at first sight, very much the appearance of a spermatozoon (figs. 23, 24).

In some cases it seems that when this process is going forward, the extra-nuclear centrosome having divided, one half of this structure passes down the stalk of the protoplasmic club and enters the rounded mass. In any case, however, at the time the extra-nuclear centrosome has become detached, a new centrosome becomes visible in the remaining round cell (figs. 23, 24, 25). From this there grows out an exceedingly fine fibre, which is much more delicate than the ordinary flagellum (figs. 23, 24, 25). The new extra-nuclear centrosome divides, and after a time a second flagellum grows out from the second extra-nuclear centrosome contained in the round form (figs. 25, 27). One peculiarity in relation to these new flagella is their great length, this often being in the proportion of 7 : 4 when contrasted with the length of the flagellum of an ordinary trypanosome.

The changes we have just described appear ordinarily to take place before the death of rats infected with *T. equiperdum*, but may be encountered also at, and for some time after, death, from which facts it might be natural to suppose that they are related to the changed condition occurring during the approach of death.

We have, however, found no evidence for this supposition. In none of the changes during the production of the round forms or latent bodies is there the least suggestion of degeneration. The growth of the new flagella and the division of the extra-nuclear centrosomes in the round forms are entirely against such a view. This latter conception is confirmed by many experiments we have made. Thus, if a rat be killed at the time the body derived from the extra-nuclear centrosome is passing towards the nucleus in large numbers of the trypanosomes, no changes analogous to those we have described take place in the blood of the dead animal. We have encountered in such cases, as time goes on, nothing but degeneration and disintegration of the trypanosomes. Again, in many cases the infection of dourine kills the rats before the life-cycle in the parasites has reached the point at which the interaction between the extra-nuclear centrosome and the nucleus occurs, and here also it is found that after death no changes take place in the trypanosomes other than those related to their degeneration, or in any way corresponding to the formation of the latent forms.

Still further, we have at various periods of the infections abstracted blood, and watched the condition of the parasites until degeneration is becoming general, and in these cases also have encountered nothing comparable to the changes we have described in relation to the formation of the latent bodies. The change is thus related to a particular stage of the development of the trypanosomes in the blood.

It will be remembered that the formation of the latent bodies in *T. gambiense* takes place at, and immediately after, the periods of maximum number of the parasites in the blood, and the immergence from the latent bodies once more occurs a considerable time later. The infection with *T. equiperdum* in rats only progresses to a first maximum, during which the rat dies. We find also that it is only in those rats which have resisted the infection for a sufficient period that the formation of latent bodies in large numbers takes place. It should, however, be pointed out that, even on the second day after the appearance of the trypanosomes in a rat infected with dourine, a few trypanosomes with club-shaped projections, and a few latent bodies with their long, fine flagella, may occasionally be encountered. This corresponds to the occasional appearance of latent bodies during almost all the periods in an infection of *T. gambiense.*

It would be extremely interesting to ascertain what exactly happens during the successive periods of maxima and minima, which succeed one another when a horse is infected with dourine; but we have found that even at the maxima of such infections the parasites are so few in number as to render it practically impossible to utilise horses for this object.

It would seem, then: that during the infection of rats with dourine, that is to say, with a form of trypanosome which under normal circumstances is not related to two distinct hosts, there exists a life-cycle among the parasites closely analogous to that occurring during the successive positive and negative periods of infection of the same animals with *T. gambiense.*

The parasites, after introduction into a rat, multiply by longitudinal fission, accompanied by amitotic division of the nucleus. After this process, an interaction takes place between the extra-nuclear centrosome and the nucleus (sexual stage ?). Division again proceeds, and finally the trypanosomes are converted into round bodies, which correspond to the latent bodies of *T. gambiense,* but possess two long and delicate flagella.

DESCRIPTION OF FIGURES.

In both plates the coloured figures are stained with Breinl's stain, and the extra-nuclear centrosome should be purple, but not so red as the intra-nuclear centrosome.

PLATE 8.

FIGS. 1—5.—Stages in the longitudinal fission of *T. equiperdum.* Figs. 1—4 showing the amitotic division of the nucleus, *n. c*, the intra-nuclear centrosome. In figs. 2 and 3 the intra-nuclear centrosome is dividing, below are the trophic granules. Figs. 1—4 are stained with Breinl's stain, fig. 5, a late stage in the fission, shows the characteristic inequality in the size of the resulting cells.

FIGS. 6—10.—Successive stages in the detachment of a portion of the nuclear substance. Figs. 7—10 stained with iron heamatoxylin. By this method the trophic granules are not shown.

FIGS. 11—15.—Successive stages of the passage of a portion of the extra-nuclear centrosome to the nucleus.

PLATE 9.

FIG. 16.—Late stage during the passage of the extra-nuclear centrosome to the nucleus. The detached mass is now practically fused with the nucleus. Compare figs. 14 and 15.

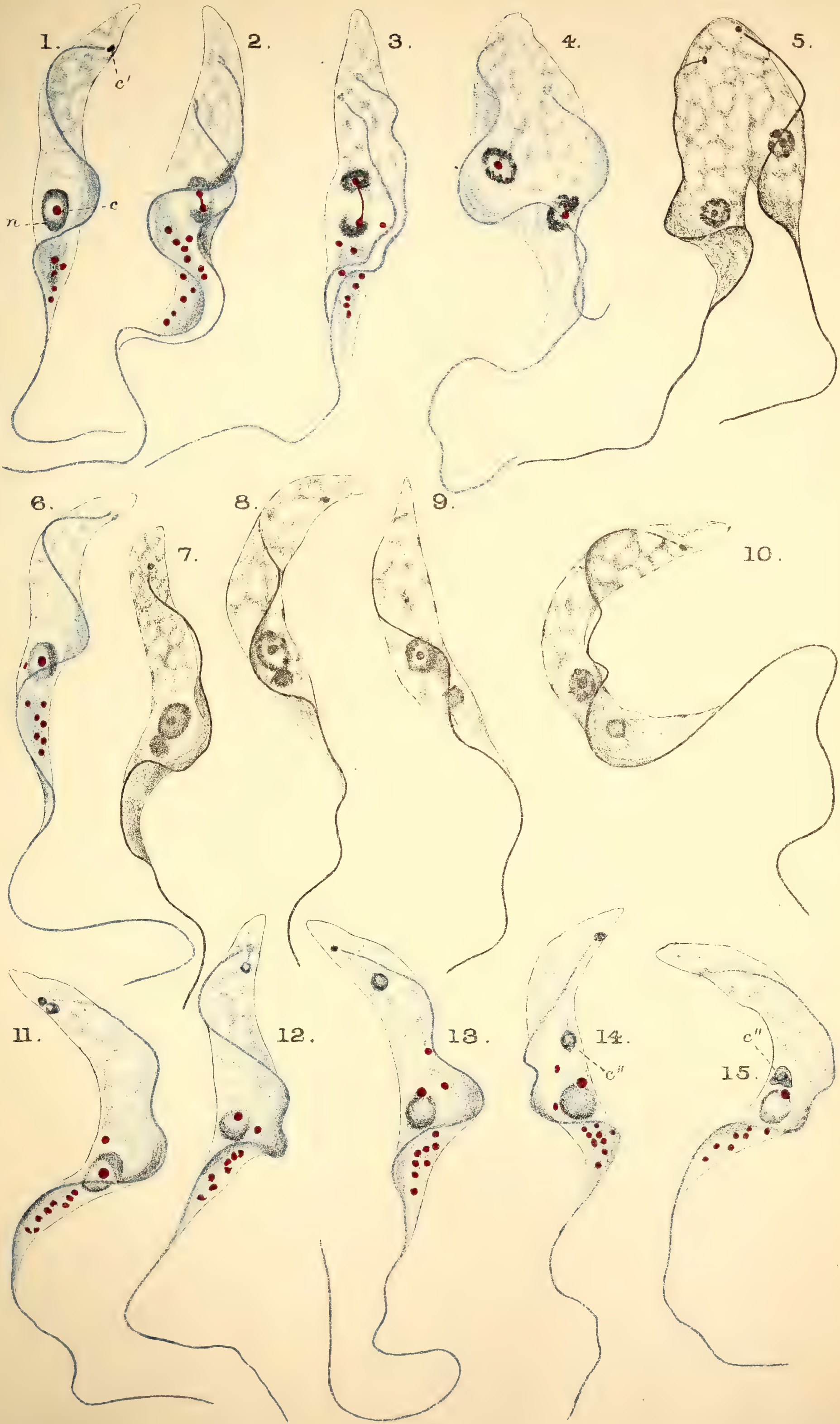
FIG. 17.—*T. equiperdum* again dividing after the fusion of a portion of the extra-nuclear centrosome with the nucleus.

FIGS. 18—22.—Stages in the development of the latent body.

FIGS. 23 and 24.—Detachment of the old flagellum and appearance of a new extra-nuclear centrosome, and a new flagellum.

FIGS. 25—28.—Complete latent bodies showing division of the extra-nuclear centrosome and the formation of two long delicate flagella.

Moore & Breinl. *Roy. Soc. Proc. B, Vol. 80, Pl. 8.*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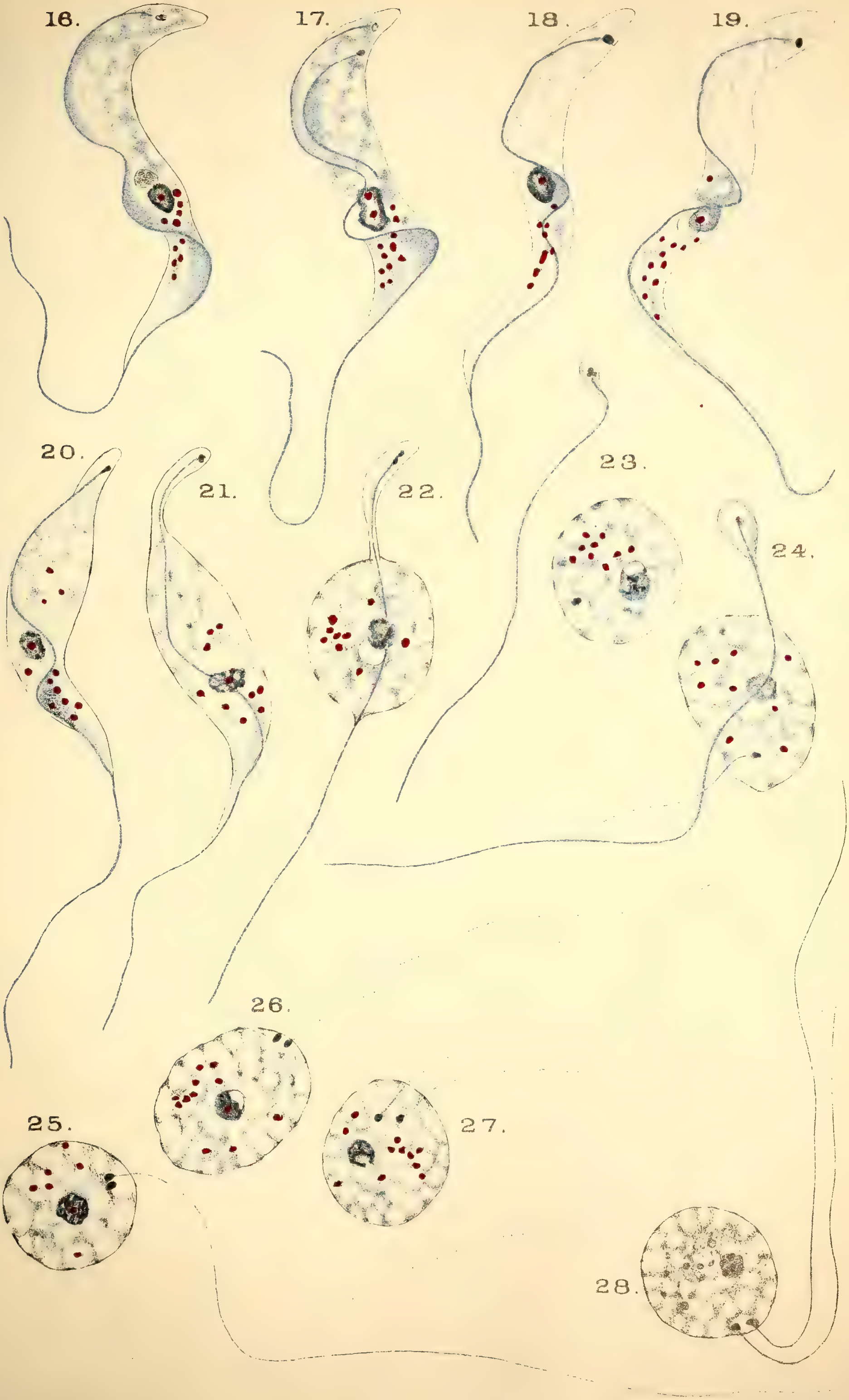
Moore & Breinl. *Roy. Soc. Proc. B, Vol. 80, Pl. 9.*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The Alcoholic Ferment of Yeast-juice. Part III.—*The Function of Phosphates in the Fermentation of Glucose by Yeast-juice.*

By Arthur Harden and William John Young (Biochemical Laboratory of the Lister Institute of Preventive Medicine).

(Communicated by C. J. Martin, F.R.S. Received March 6,—Read April 2, 1908.)

In a previous communication the authors have shown* that when a soluble phosphate is added to a fermenting mixture of glucose and yeast-juice the following phenomena are to be observed: (1) The rate of fermentation is at once greatly increased. (2) This acceleration lasts for a short time and the rate then falls off, and returns approximately to its original value. (3) During this period the extra amount of carbon dioxide evolved and alcohol produced are equivalent to the phosphate added. (4) The phosphate is converted into a form which is not precipitable by magnesia-mixture, and is then probably present as a salt of a hexosephosphoric acid.

(1) *Effect of the Addition of Phosphate on the Total Fermentation.*

The addition of phosphate, however, does not simply produce this initial decomposition of an equivalent of glucose, but also, as a rule, a greater total fermentation, after allowance has been made for the amount decomposed during the initial period.

This is clearly shown by the results embodied in the following table.

In each experiment two or more portions of 25 c.c. of yeast-juice were taken, a solution of glucose alone, or one of glucose and phosphate, added, and the total volume made to 50 c.c. The solution of phosphate employed had a concentration of about 0·3 molar, and the concentration of glucose was 20 grammes per 100 c.c. in experiment 8, and 10 grammes per 100 c.c. in all the others. The fermentation was carried out at 25° in presence of toluene until the evolution of gas ceased. The numbers in the last column show the increase in the total fermentation produced during the period subsequent to the initial acceleration.

It will be seen that the increase which occurs after the initial period varies from about 10 per cent. of the original fermentation to as much as 150 per cent.

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* 'Roy. Soc. Proc.,' B, vol. 77, 1906, p. 405.

Experiment.	CO_2 evolved without phosphate.	Cubic centimetres of phosphate solution added.	CO_2 evolved in presence of phosphate.	Increase due to phosphate.	CO_2 equivalent to phosphate.	Increase after initial period.
	grammes.	c.c.	grammes.	gramme.	gramme.	gramme.
1	0·484	10	0·717	0·233	0·132	0·101
2	1·280	5	1·584	0·304	0·066	0·238
3*a*	0·422	10	0·634	0·212	0·132	0·080
3*b*	0·422	20	0·748	0·326	0·264	0·062
4	0·440	10	1·258	0·818	0·132	0·686
5	0·405	5	0·515	0·110	0·070	0·040
6	0·603	5	0·735	0·132	0·066	0·066
7	0·438	5	0·593	0·155	0·057	0·098
8	1·016	15	1·632	0·616	0·198	0·418
	c.c.		c.c.	c.c.	c.c.	c.c.
9	369	10	629	260	63	197
10	337	10	569	232	56	176

(2) *Recurrence of Phosphate.*

The reason for this increase in the amount of sugar decomposed in the long period following the short initial period of acceleration appears to be that the phosphorus compound first formed, which is a hexosephosphate of the formula $C_6H_{10}O_4(PO_4R_2)_2$,* is slowly hydrolysed, probably by an enzyme, with the production of a phosphate and a hexose. The phosphate is thus slowly regenerated and then again undergoes the reaction, causing an increased fermentation in the same manner as when it was originally added.

This recurrence of phosphate is clearly shown by the following experiment. A known amount of phosphate was added to yeast-juice containing glucose, and the mixture incubated at 25° with toluene. At the close of the initial period a sample was removed, boiled and filtered, and the free and total phosphate present in it estimated, and this process was repeated at stated times. The results obtained are given below, the amounts of phosphate being expressed in grammes of $Mg_2P_2O_7$ per 10 c.c.

Experiment 11.—215 c.c. of yeast-juice + 20 grammes of glucose were made to 375 c.c. with a solution of potassium phosphate, the amount of the latter being equivalent to 0·133 gramme $Mg_2P_2O_7$ per 10 c.c. of the resulting liquid.

The slight increase in the total phosphate present is due to a corresponding degree of evaporation during the experiment. It will be seen that the free phosphate per 10 c.c. gradually increases from 0·021 to 0·226, so that 0·205 gramme is regenerated. Since the total phosphorus, expressed as phosphate in

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* Young, 'Chem. Soc. Proc.,' 1907, vol. 65.

Time in hours.	Free phosphate as $Mg_2P_2O_7$ per 10 c.c.	Total phosphate as $Mg_2P_2O_7$ per 10 c.c.
	gramme.	gramme.
5·5	0·021	0·266
18·0	0·093	0·269
66·0	0.133	
138·0	0·175	
426·0	0·226	0·273

the original juice, was 0·266−0·133=0·133 gramme, it follows that at least 0·205−0·133=0·072 gramme of this has been derived from the hexosephosphate produced during the initial period from the added phosphate.

Even in the absence of added phosphate a gradual production of free phosphate occurs when yeast-juice is incubated at 25°. In the absence of glucose, when the fermentation is very small, the increase in the amount of free phosphate is comparatively rapid, whereas, in the presence of glucose, the fermentation lasts for a considerable period, and the appearance of free phosphate is delayed, since it continually enters into fresh combination as rapidly as it is formed.

Examples of this are the following. The various materials were digested at 25° with toluene for the time shown, and were then boiled and filtered and the free phosphate estimated.

Experiment.	Material digested.	Time of digestion.	Free phosphate as $Mg_2P_2O_7$ per 25 c.c.
		days.	gramme.
12	(*a*) Yeast-juice alone	0	0·128
	(*b*) " "	2	0·284
	(*c*) " "	10	0·283
	(*d*) Yeast-juice + glucose	2	0·123
	(*e*) " "	10	0·255
13	(*a*) Yeast-juice alone	0	0·024
	(*b*) " "	1	0·053
	(*c*) " "	2	0·063
	(*d*) " "	4	0·069
	(*e*) Yeast-juice + glucose	1	0·011
	(*f*) " "	2	0·025
	(*g*) " "	4	0·051
		hours.	
14	(*a*) Yeast-juice + glucose + phosphate (0·276 gramme per 25 c.c.)	1·25	0·063
	(*b*) " " "	46·0	0·138
	(*c*) " " "	96·0	0·387
	(*d*) " " "	168·0	0·453
	(*e*) " " "	240·0	0·489
	(*f*) " " "	336·0	0·501
	(*g*) " " "	456·0	0·523

In this last experiment (No. 14) sample (*a*) was taken at the close of the initial period and sample (*b*) immediately after the cessation of fermentation, these points being determined by observation of another sample of the yeast-juice.

It is to be noted that during the fermentation only a small increase occurs in the amount of free phosphate (0·075 gramme), while after the cessation of fermentation the increase amounts to about three times as much (0·249 gramme) in approximately an equal time. The total phosphate of the original juice was 0·350 gramme per 25 c.c. and the amount added was 0·276 gramme. Since only 0·104 gramme of phosphate remains combined at the close of the experiment, it follows that at least 0·276 − 0·104 = 0·172 gramme has been regenerated from the hexosephosphate.

The recurrence of phosphate in these cases appears to be due to the action of an enzyme—whether a special enzyme or one of those already known to occur in yeast-juice has not yet been determined.

After yeast-juice containing the sodium salt of hexosephosphoric acid has been boiled the amount of free phosphate remains practically unaltered when the liquid is incubated at 37°, as is shown by the following experiment.

Experiment 15.—To yeast-juice containing glucose and an amount of free phosphate corresponding to 0·184 gramme of $Mg_2P_2O_7$ per 25 c.c. was added sodium phosphate equivalent to 0·285 gramme of $Mg_2P_2O_7$ and the mixture incubated at 37° in presence of toluene. A sample was taken at the close of the initial period, and as soon as fermentation had almost ceased the whole was boiled, the free phosphate estimated and the remainder of the boiled liquid preserved at 37°, samples being taken at intervals.

At the close of the initial period, 25 c.c. yielded only 0·04 gramme of $Mg_2P_2O_7$, so that practically the whole of the added phosphate must have been converted into a salt of hexosephosphoric acid. At the cessation of fermentation the same volume yielded 0·131 gramme of $Mg_2P_2O_7$, so that at least 0·15 gramme was still present as a hexosephosphate.

After incubation of the boiled liquid at 37° for an additional 144 hours, the amount of free phosphate had only increased to 0·138 gramme, so that the action practically ceased when the liquid was boiled.

(3) *Nature of the Chemical Change which occurs in the Fermentation of Glucose by Yeast-juice.*

The cycle of changes which is undergone by a phosphate in the presence of yeast-juice and glucose appears from the foregoing to be as follows :—

(1) $2C_6H_{12}O_6 + 2R_2HPO_4 = 2CO_2 + 2C_2H_6O + C_6H_{10}O_4(PO_4R_2)_2 + 2H_2O.$

(2) $C_6H_{10}O_4(PO_4R_2)_2 + 2H_2O = C_6H_{12}O_6 + 2R_2HPO_4.$

The first of these equations does not include the fermenting complex, without which, however, the change does not occur, and it is probable that both the glucose and the phosphate form an intermediate association with this complex, which then breaks down, giving rise to the substances on the right-hand side of the equation, and at the same time regenerating the fermenting complex.

Since free phosphate and a hexosephosphate are invariably present in the yeast-juice prepared by grinding yeast, it follows that at all events some portion of the fermentation is always due to the foregoing reactions. During the initial period of rapid fermentation, as long as free phosphate is still present, the greater part of the change is certainly due to this reaction, whilst in the succeeding period of slower fermentation the constant production of free phosphate by the enzymatic hydrolysis of the hexosephosphate already formed, or by the action of proteoclastic enzymes on phosphoproteins, renders it equally certain that some portion of this greatly diminished fermentation must also be ascribed to the same reaction.

The question at once arises whether it is not possible that the whole of the fermentation is due to this reaction. So far, no fact has been encountered which is inconsistent with this view. In previous communications* the authors have shown that at least two substances are concerned in the production of carbon dioxide and alcohol from glucose: the ferment, which is thermolabile, and the coferment, which is thermostable; and, further, that phosphates are incapable of producing any fermentation when they are added to a mixture of the ferment and glucose in the absence of the coferment.

If the theory suggested above be found to be correct, it will be necessary to complete this statement. Two possibilities present themselves. Either a third substance, a soluble phosphate, is also necessary, and in the presence of the fermenting complex, made up of ferment and coferment, undergoes the reaction under discussion; or the coferment may itself be a complex substance containing a group of unknown composition, united with the phosphoric acid group. The latter would then be passed on to the glucose during fermentation, and a new group taken up from the phosphate, a continuous conversion of phosphate into hexosephosphate being thus effected.

If this cycle of changes correctly represents the reaction which occurs, it follows that the rate of fermentation after the initial period of acceleration depends, in the first instance, on the rate at which phosphate is liberated according to equation (2).

The most satisfactory method of testing the accuracy of this view would be to free both ferment and coferment from phosphate and materials capable of yielding phosphates under the influence of the enzymes which are present, and then to ascertain whether the mixture of these purified materials would ferment glucose; and, further, if they did not, whether the addition of a phosphate would bring about fermentation. Many attempts have been made to realise these conditions, but hitherto without success. It is obvious that if the coferment be a hydrolysable derivative of phosphoric acid, as suggested above, success in this direction cannot be anticipated.

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* 'Roy. Soc. Proc.,' B, vol. 77, 1906, p. 405; B, vol. 78, 1906, p. 369.

Some idea of the extent to which alcoholic fermentation is due to this recurrence of phosphate could also be gained by ascertaining the amount of free phosphate produced from hexosephosphate in yeast-juice in a given time in the absence of glucose, and comparing this with the amount of carbon dioxide evolved in the presence of glucose under otherwise identical conditions.

In practice, however, two difficulties present themselves. In the first place, in the absence of fermentable sugar, or when the concentration of this is very low, free phosphate accumulates, and the rate of hydrolysis of the hexosephosphate is thus diminished; whereas in the presence of glucose the concentration of phosphate remains constant at a low value during fermentation, and the rate of hydrolysis of hexosephosphate accordingly remains at its maximum for a considerable period.

This inhibitory effect of phosphate on the hydrolysis of hexosephosphate in the absence of all fermentation is shown in the following experiment, which also indicates the extent to which this hydrolysis occurs under these conditions.

Experiment 16.—In order to avoid all fermentation, the inactive residue obtained by filtering yeast-juice through a Martin gelatin-filter was employed, and equal weights of this were incubated for 20 hours with: (1) water; (2) a solution of sodium hexosephosphate free from glucose and free phosphate; (3) a solution of sodium hexosephosphate + an equivalent amount of sodium phosphate. A solution of hexosephosphate was also incubated alone, the conditions of concentration and alkalinity being identical in all four solutions. At the expiration of 20 hours, the solutions were all boiled, and the free phosphate in the filtrates estimated. In the following table the numbers represent the weight of $Mg_2P_2O_7$ found.

The numbers in the last column are obtained by adding together the amount of phosphate produced separately by the incubation of the hexosephosphate and the residue, and subtracting their sum from the free phosphate produced in the other two solutions.

It appears from this that in this particular instance the enzymatic

Solution.	Amount of free phosphate present.		Amount of hexosephosphate hydrolysed by enzyme.
	Before incubation.	After incubation.	
1. Hexosephosphate equivalent to 0·1626 gramme $Mg_2P_2O_7$	0·0	0·0089	
2. 0·75 gramme residue + water ……	0·0	0·0166	
3. 0·75 gramme residue + hexosephosphate equivalent to 0·1626 gramme $Mg_2P_2O_7$	0·0	0·1068	0·0813
4. 0·75 gramme residue + hexosephosphate as above, + sodium phosphate equivalent to 0·1626 gramme $Mg_2P_2O_7$	0·1626	0·1884	0·0003

decomposition of the hexosephosphate is almost completely arrested by the presence of an equivalent of free phosphate.

The amount of phosphate actually produced in solution 3 from all sources is 0·1068 gramme $Mg_2P_2O_7$, which corresponds to an evolution of about 22 c.c. of carbon dioxide, and this in spite of the fact that the phosphate has been allowed to accumulate. The amount which would be evolved in presence of glucose and coferment would naturally exceed this, owing to the continued reconversion of the phosphate into hexosephosphate.

This experiment also shows that the ferment which brings about the hydrolysis of the hexosephosphate is present in the residue obtained by filtering yeast-juice through a Martin-filter, and does not require a dialysable coferment.

The second difficulty arises from the fact that when yeast-juice or a mixture of ferment and coferment is employed, a certain amount of fermentation always occurs, even in the absence of added sugar. This is due to sugar formed in the liquid, in part by the hydrolysis of glycogen and dextrins and in part by the hydrolysis of the hexosephosphate itself, which yields a fermentable sugar as one of its products. The practical result is that if an actual comparison be instituted between the production of phosphate in the absence of added glucose and the evolution of carbon dioxide in the presence of glucose, it will be necessary to take the sum of the carbon dioxide actually evolved and the carbon dioxide equivalent of the phosphate produced in the absence of added glucose, and this will always be found to be less than the volume of carbon dioxide observed in the presence of glucose. The result of such a comparison is shown in the two following experiments.

A mixture was made of yeast-juice with a solution containing a suitable amount of sodium phosphate and just sufficient glucose to bring about the conversion of the greater portion of the phosphate into hexosephosphate, this being found by experiment to be rather more than an equivalent of glucose. The mixture was incubated until this conversion had been accomplished and the rate of fermentation had become steady. A sample was then taken and boiled and the free phosphate estimated in the filtrate. The remainder was incubated for a further period, the evolution of gas being noted, and a second sample was then taken.

A parallel experiment was carried out with yeast-juice containing the same amount of sodium phosphate in presence of 10 grammes of glucose per 100 c.c., and the evolution of gas during the same period was observed. The solutions used and the results obtained were as follows :—

Experiment 17.—(1) 100 c.c. yeast-juice + 30 c.c. of a solution containing 1·3 grammes sodium phosphate and 2·62 grammes glucose. (2) 100 c.c. yeast-juice + 30 c.c. of a solution containing 1·3 grammes sodium phosphate and 13 grammes glucose.

Experiment 18.—(1) 100 c.c. yeast-juice + 40 c.c. of a solution containing 1·7 grammes sodium phosphate and 3·16 grammes glucose. (2) 100 c.c. yeast-juice + 40 c.c. of a solution containing 1·7 grammes sodium phosphate and 14 grammes glucose.

Experiment.	Solution 1 (low concentration of glucose).						Solution 2 (excess of glucose).
	Original phosphate.	Final phosphate.	Phosphate produced.	CO_2 equivalent.	CO_2 evolved.	Total CO_2 equivalent.	CO_2 evolved.
				c.c.	c.c.		
17	0·0559	0·1052	0·0493	10·7	39·8	50·5	93·9
18	0·1036	0·1701	0·0665	14·6	27·7	42·3	69·2

These experiments show that although the sum of the carbon dioxide evolved and that equivalent to the phosphate produced in absence of glucose is invariably less than the amount of carbon dioxide evolved in the presence of glucose, yet the difference is no greater than might be expected from a knowledge of the prevailing conditions, and is quite consistent with the view that the whole of the fermentation proceeds according to the equation proposed.

(4) *Influence of Concentration of Phosphate on the Course of the Fermentation.*

When a phosphate is added to a fermenting mixture of glucose and yeast-juice, the effect varies both with the concentration of the phosphate and with the particular specimen of yeast-juice employed. With low concentrations of phosphate the acceleration produced is so transient that no accurate measurements of rate can be made. As soon as the amount of phosphate added is sufficiently large, it is found that the rate of evolution of carbon dioxide suddenly increases from 5 to 10 times, and then rapidly falls approximately to its original value.

As the concentration of phosphate is still further increased, it is first observed that the maximum velocity, which is still attained immediately on the addition of the phosphate, is maintained for a certain period before the fall commences, and then, as the increase in concentration of phosphate proceeds, that the maximum is only gradually attained after the addition, the period required for this increasing with the concentration of the phosphate. Moreover, with these higher concentrations the maximum rate attained is less than that reached with lower concentrations, and, further, the rate falls off more slowly. The concentration of phosphate which produces the highest rate, which may be termed the optimum concentration, varies very considerably with different specimens of yeast-juice.

All these points are illustrated by the accompanying tables and curves.

Experiment 19.—The following solutions were employed:—

(1) 25 c.c. yeast-juice + 15 c.c. sodium bicarbonate solution.

(2) 25 c.c. yeast-juice + 10 c.c. of potassium phosphate solution (0·3 molar) + 5 c.c. sodium bicarbonate solution.

(3) 25 c.c. yeast-juice + 15 c.c. potassium phosphate solution. All the solutions contained 4 grammes of glucose, and the experiment was carried out at 25° in the presence of toluene. The solution of bicarbonate added contained an amount of this salt equal to that formed by the action of carbon dioxide on an equal volume of the solution of potassium phosphate employed.

Time after addition in minutes.	Carbon dioxide evolved in preceding 5 minutes with *n* cubic centimetres of 0·3 molar potassium phosphate added.		
	n = 0 c.c.	*n* = 10 c.c.	*n* = 15 c.c.
5	4·0	11·1	7·7
10	3·2	16·0	9·7
15	4·2	20·2	12·1
20	3·6	**22·4**	16·1
25	4·3	17·4	18·4
30	3·6	6·6	19·4
35	4·3	4·6	**20·4**
40	3·2	4·7	16·7
45	—	4·5	12·7
50	—	4·2	6·0
55	—	4·1	4·0

Experiment 20.—

(1) 25 c.c yeast-juice + 5 c.c. phosphate + 15 c.c. bicarbonate.
(2) 25 c.c. „ + 10 c.c. „ + 10 c.c. „
(3) 25 c.c. „ + 15 c.c. „ + 5 c.c. „
(4) 25 c.c. „ + 20 c.c. „ + 0 c.c. „

The concentration of glucose was 4·5 grammes per 45 c.c., and the experiment was carried out at 25° in presence of toluene.

Time after addition in minutes.	Carbon dioxide evolved in preceding 5 minutes with *n* cubic centimetres of 0·3 molar phosphate added.			
	n = 5 c.c.	*n* = 10 c.c.	*n* = 15 c.c.	*n* = 20 c.c.
5	7·1	2·7	1·6	1·6
10	12·7	6·2	2·4	1·7
15	**16·3**	8·1	3·0	2·0
20	7·7	9·6	3·3	2·0
25	1·9	12·4	4·0	2·3
30	1·8	14·5	5·1	2·6
35	—	**16·8**	6·0	2·7
40	—	15·0	6·5	3·1
45	—	2·9	7·3	3·5
50	—	1·7	8·5	3·8
55	—	—	**8·7**	4·4
60	—	—	8·5	4·9
65	—	—	8·6	5·6
70	—	—	8·1	5·7
75	—	—	7·6	5·6
80	—	—	7·0	**6·0**
85	—	—	6·0	6·0
90	—	—	—	6·0
95	—	—	—	5·5
100	—	—	—	5·4
105	—	—	—	5·0

Curves A, B, C, and D (fig. 1) show the rate of evolution per five minutes for the four solutions in experiment 20. The time of addition is taken as zero, the rate before addition being constant, as shown in the curves.

It will be observed that in experiment 19 practically the same maximum is attained with 10 and 15 c.c. of phosphate, whereas, in experiment 20, 5 and 10 c.c. give the same maximum, whilst 15 c.c. produce a much lower maximum, and 20 c.c. a still lower one, the rate at which the velocity diminishes after the attainment of the maximum being correspondingly slow in these last two cases. By calculating the amount of phosphate which has disappeared as such from the amount of carbon dioxide evolved, it is found that the maximum does not occur at the same concentration of free phosphate in each case.

These results suggest that the phosphate is capable of forming two or

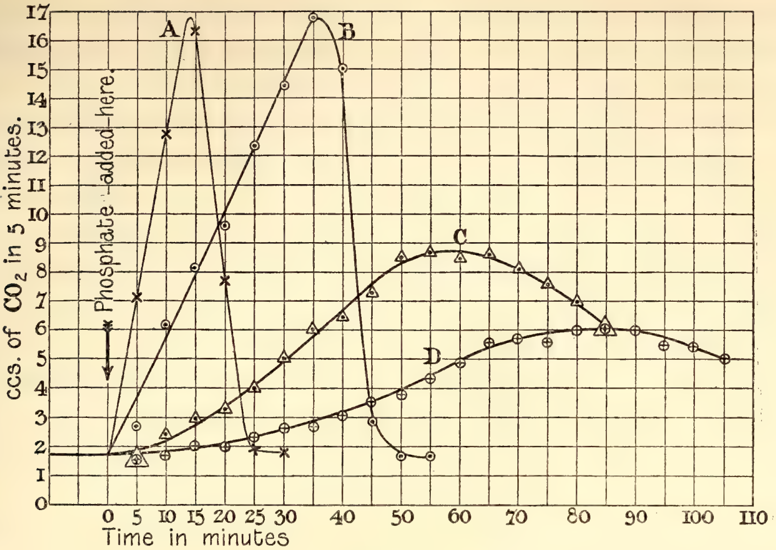

Fig. 1.

more different unstable associations with the fermenting complex. One of these, formed with low concentrations of the phosphate, has the composition most favourable for the decomposition of sugar, whilst the others, formed with high concentrations of phosphate, contain more of the latter, probably associated in such a way with the fermenting complex as to render the latter partially or wholly incapable of effecting the decomposition of the sugar molecule. As the fermentation proceeds slowly in the presence of excess of phosphate, the concentration of the latter is reduced by conversion into hexosephosphate, and a redistribution of phosphate occurs, resulting in the gradual change of the less active into the more active association of phosphate with fermenting complex, and a consequent rise in the rate of fermentation.

In those cases in which the maximum rate corresponding to the optimum concentration of phosphate is never attained, some secondary cause may be supposed to intervene, such as a permanent change in a portion of the fermenting complex, accumulation of the products of the reaction, etc. Experiments on this point are being carried out by varying independently the concentration of the ferment, the coferment, the phosphate, and the hexosephosphate.

In agreement with these conclusions it is found that a high rate of evolution of carbon dioxide can be maintained for a considerable period by the gradual addition of phosphate in such a way that the concentration of free phosphate remains approximately at the optimum value. This may be effected by adding in every interval of five minutes the amount of phosphate equivalent to the carbon dioxide evolved in excess of the normal rate of fermentation of the yeast-juice during the same interval of time.

Experiment 21.—25 c.c. of yeast-juice + 2·5 grammes glucose were incubated at 25° in the presence of toluene until the rate became constant. A solution of potassium phosphate (2 mol. K_2HPO_4 and 1 mol. of KH_2PO_4) of 0·3 molar concentration was then gradually added from a graduated pipette provided with a tap and passing through the cork of the fermentation flask. Column 2 shows the c.c. of this added in each period of five minutes, and column 3 the evolution of carbon dioxide during each period of five minutes. The normal rate of the yeast-juice, in the absence of added phosphate, was 3 c.c. per five minutes. It will be seen that an average rate of about 15 was maintained for an hour and a quarter, 32 c.c. of the phosphate solution being added in all.

1. Time in minutes.	2. Cubic centimetres of phosphate solution added in each 5 minutes.	3. Evolution of carbon dioxide in each 5 minutes.
	c.c.	
5	5·0	3·1
10	1·5	15·4
15	2·0	16·2
20	1·0	20·2
25	2·5	17·2
30	1·5	12·7
35	2·0	15·7
40	0·0	17·4
45	0·0	9·6
50	3·0	8·4
55	3·0	15·4
60	3·0	20·2
65	3·0	17·2
70	3·0	14·8
75	1·5	14·9

Further experiments on this subject are in progress, particularly with respect to the relations between phosphate and coferment, and the bearing of these phenomena on the fermentation of sugars by living yeast.

Summary.

1. The addition of a phosphate to a fermenting mixture of glucose and yeast-juice not only produces a temporary acceleration in the rate of fermentation, but, in addition to this, an increased total fermentation.

2. This last effect is due to the fact that the hexosephosphate formed during

the period of temporary acceleration is continually hydrolysed by an enzyme, with production of free phosphate, which again enters into reaction and thus brings about an increased fermentation.

3. It appears probable that the presence of phosphate is essential for the alcoholic fermentation of glucose by yeast-juice, the reaction which occurs being the following :—

(1) $2C_6H_{12}O_6 + 2R_2HPO_4 = 2CO_2 + 2C_2H_6O + C_6H_{10}O_4(PO_4R_2)_2 + 2H_2O.$

This reaction is only realised in the presence of the ferment and coferment discussed in previous communications, phosphate alone being unable, in the absence of coferment, to bring about fermentation in a mixture of ferment and glucose.

The hexosephosphate thus formed is then hydrolysed :

(2) $C_6H_{10}O_4(PO_4R_2)_2 + 2H_2O = C_6H_{12}O_6 + 2R_2HPO_4.$

The rate at which this second reaction occurs determines the rate of fermentation observed when glucose is fermented by yeast-juice.

4. An optimum concentration of phosphate exists which produces a maximum initial rate of fermentation. Increase of concentration beyond this optimum diminishes the rate of fermentation.

Studies on Enzyme Action. XI.—*Hydrolysis of Raffinose by Acids and Enzymes.*

By H. E. Armstrong, F.R.S., and W. H. Glover, Ph.D.

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Subject slips :—

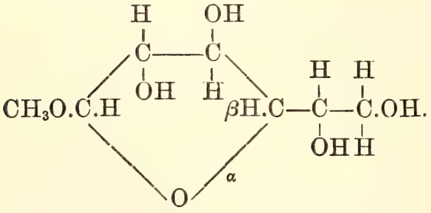
D 1820	Cane-sugar. Hydrolysis by acids and enzymes.
D 1830	Raffinose. Hydrolysis by acids and enzymes.
D 8014	Invertase, action of, on raffinose and sucrose compared.
D 7095	Cane-sugar and raffinose, hydrolysis of, by acids and enzymes compared.
Q 1240	Invertase, action of, on raffinose and sucrose compared.]

In a previous communication of this series (vol. 74, p. 191),* it is pointed out that the various glucosides are hydrolysed by acids at very different rates, the relative values being approximately of the order shown in the following table :—

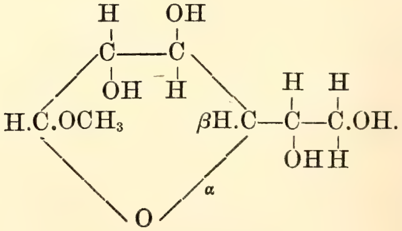
α-Methylglucoside	100
β- „	180
α-Methylgalactoside	540
β- „	880
Salicin (a β-glucoside)	600
Milk-sugar (a β-galactoside)......	720
Maltose (an α-glucoside)	740

Cane-sugar, it is to be remembered, is hydrolysed at a rate vastly more rapid—at least 1000 times as rapidly as maltose, in fact. These differences, taking into account the peculiar specific behaviour of enzymes as hydrolytic agents, raise questions of interest from the chemical side and they are of no slight significance perhaps also from a biological point of view.

The two stereo-isomeric methylglucosides are represented by the following formulæ :—



α-Methylglucoside



β-Methylglucoside

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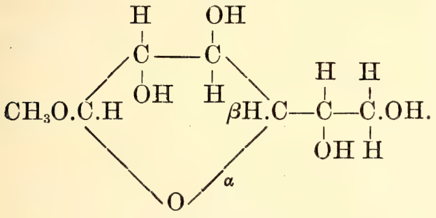
* All references are to these 'Proceedings.'

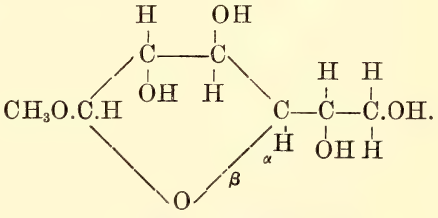
It will be seen that the two radicles H and OCH_3 shown as attached to the one carbon atom merely occupy different or reversed positions relatively to the two other radicles with which the carbon atom is connected.

As to the manner in which the hydrolytic attack takes place, two views are possible: the one being that the compound behaves much as the simple ether $CH_3.O.CH_3$ would and that the hydrolyst becomes associated with the oxygen atom to which the CH_3 group is attached; the other being that the attachment is to the oxygen atom in the ring. On the former view, it is to be supposed that the two isomeric compounds would be hydrolysed with equal readiness, as the CH_3O groups are equally weighted; but on the latter, it is conceivable that the methoxy-group is less easily accessible to hydrolysis in the one case than it is in the other; or it may be that an *inductive effect* is exercised by the one oxygen atom upon the other which renders the oxygen atom in the ring either more or less attractive of the hydrolyst, as the case may be.

In the galactosides, the CH_3O group occupy the same relative positions as in the two glucosides; but the contiguous oxygen atom in the pentaphane ring must be supposed to occupy a position slightly different from that which it occupies in glucose, its connection with the carbon atom on the right being slightly different, as shown in the two following formulæ:—



a-Methylglucoside



a-Methylgalactoside

Apparently, seeing that the galactosides are the more easily hydrolysed, this difference is sufficient to render the hydrolyst—the attack of which in all probability is directed from the oxygen atom in the ring—more accessible to the neighbouring CH_3O group than it is in the glucosides. The behaviour of the glucosidic acetates appears to be in accordance with such an interpretation.* In models of glucose and galactose constructed with tetrahedra to represent the carbon atoms, if these atoms are arranged in a vertical plane the oxygen atom in the ring occupies somewhat different positions more or less outside this plane; the difference in the relative positions of the OCH_3 group and the oxygen atom in the ring, according as the position of these on either side of the ring plane is varied, then becomes apparent.

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* *Cf.* Armstrong, E. F., and Arup, 'Chem. Soc. Trans.,' 1904, vol. 85, p. 1043.

There can be little doubt that considerations of the order here pictured are more or less applicable to the explanation of differences such as those under discussion and that it may be possible eventually, proceeding on these lines, to discriminate between alternative formulæ applicable to compounds such as glucose and galactose. It is from this point of view that the study of hydrolytic changes is of supreme importance.

Enzymes probably act much in the same way as acid hydrolysts; the attachment of the enzyme, however, appears to be more general and thorough, so to speak, than that of the acid hydrolyst and presumably extends over a large part of the molecule (compare III and X, B, vol. 79, p. 361). But in both cases the attack is directed, it may be supposed, from the oxygen atom in the pentaphane ring adjoining the group which undergoes hydrolysis.

In the hope of obtaining further information bearing on this refined problem, the behaviour of raffinose towards acids and enzymes has been studied in comparison with that of cane-sugar, raffinose being a triose formed of cane-sugar *weighted* by the attachment of a molecule of galactose.

Raffinose is a reserve material which accompanies cane-sugar in the sugar beet, in cotton seed, in barley and in wheat, for example. It can either be resolved into galactose and cane-sugar by a special enzyme or it can be resolved into fructose and a biose isomeric with cane-sugar—*melibiose*—by the action of invertase, the enzyme which resolves cane-sugar into glucose and fructose.

Hydrolysis of Cane-sugar and Raffinose with the aid of Acids.

Raffinose is easily hydrolysed with the aid of acids at ordinary temperatures, the products being fructose and melibiose, the latter, like maltose and milk-sugar, undergoing change only at higher temperatures.

The experiments with acids were carried out at 25° C. in the manner described by Caldwell (A, vol. 78, p. 285), with an improved apparatus an account of which will be given at an early date by Messrs. Caldwell and Whymper. Except where stated otherwise, the polariscope readings were taken with the aid of a spectroscopic eyepiece, using a mercury lamp as the luminous source, the light being of the refrangibility of the dominant line in the green. As raffinose is less soluble than cane-sugar, solutions of quarter molecular strength were used. A complete record of two experiments is given on the left-hand side of Table I. The columns on the right contain the values of the velocity constant deduced by means of the formula

$$K = \frac{10^5}{t} \log_{10} \frac{a}{a-x} \cdot$$

Table I.—Hydrolysis of Cane-sugar and of Raffinose by Acids at 25° in Solutions containing 1000 grammes of Water + 0·25 gramme molecular proportion of Carbohydrate + 1 gramme molecular proportion of Acid.

Cane-sugar + HCl.			Raffinose + HCl.		Cane-sugar.						Raffinose.				
Volumes of the solutions in c.c. ………………					1073·8	1084·6			1092		1098·6	1108·9		1148·1	
Time.	α_{Hg}.	K.	α_{Hg}.	K.	HCl.	HNO_3.			H_2SO_4.		HCl.	HNO_3.		H_2SO_4.	
mins.															
0	7·160	—	27·388	—	—	—	—	—	—	—	—	—	—	—	—
20	4·720	502	25·503	421	500	—	465	462	551	—	424	388	393	445	444
25	4·208	499	25·101	418	499	466	461	465	552	—	418	388	392	—	442
30	3·720	498	24·700	419	498	464	461	466	551	—	422	389	391	446	—
35	3·244	499	24·327	418	497	474	459	465	548	—	420	387	392	—	450
40	2·814	498	23·970	418	504	472	463	465	551	552	420	386	391	448	—
45	2·380	500	23·630	418	499	470	460	466	550	554	423	387	394	451	447
50	2·000	498	23·280	421	498	472	459	463	548	555	423	385	391	441	442
55	1·628	498	22·700	418	502	468	464	463	546	551	420	387	393	449	447
60	1·274	499	22·146	418	496	468	464	459	545	556	420	388	397	445	444
65	0·938	499	21·651	417	500	468	463	460	550	555	419	387	394	442	446
70	0·634	498	21·184	419	503	466	463	459	546	553	418	386	395	443	447
75	0·310	502	20·747	421	503	466	462	460	546	550	419	386	395	446	445
80	0·040	501	20·407	418	504	466	466	461	545	545	418	388	395	445	447
85	−0·213	500	20·106	414	500	466	464	459	550	547	422	387	—	447	447
90	−0·492	503	19·763	417	498	466	462	460	549	550	417	386	—	448	446
95	−0·745	505	19·473	418	501	470	464	458	—	547	420	—	—	444	448
100	−0·942	502	19·200	420	500	466	463	460	—	545	417	—	—	446	448
105	−1·140	501	18·980	419	504	467	464	—	—	545	416	—	—	443	446
110	−1·350	503	—	—	502	471	462	—	—	549	415	—	—	446	446
∞	−4·659	—	16·695	—	—	—	—	—	—	—	—	—	—	—	—
Means ………		500	—	418·5	500	468	462·5	462	548·5	550	419·5	387	393	446	446

The values deduced for cane-sugar are in fair agreement with those obtained by other workers in our laboratory. Contrasting the mean values, they are as follows:—

	Nitric acid.	Chlorhydric acid.	Sulphuric acid.
Cane-sugar	464	500	549
Raffinose	390	419	446

The three acids differ in their activity towards both sugars: the cause of this difference will be discussed in a separate communication, dealing with the sucroclastic action of acids generally.

It is clear that raffinose is less easily hydrolysed than cane-sugar, at a rate nearly one-fifth less than that at which the latter undergoes change. The ratios for each acid are nearly the same in the case of nitric acid and chlorhydric acid, but sulphuric acid is relatively less active towards raffinose—a result not without interest, the significance of which, however, need not be discussed here.

Hydrolysis of Cane-sugar and Raffinose by Invertase.

In contrasting their behaviour towards invertase, comparative experiments were carried out simultaneously with the two sugars under similar conditions. A known volume of a very weak solution of invertase at 25° C. was added to a known volume of the sugar solution of definite concentration, also at 25°, contained in a Jena-glass flask; after adding a few drops of toluene, the flask was corked up and placed in an incubator kept at 25°. At stated intervals, 20 c.c. samples were withdrawn from the flask by means of a pipette and run into small Jena-glass flasks, each containing a single drop of a strong aqueous solution of sodium hydroxide. By this means the action of the enzyme was at once arrested and equilibrium established between the stereo-isomeric forms of the sugars in solution. The final values were obtained by keeping part of the solution at 25° C. during 24 hours, at the end of which time a drop of sodium hydroxide solution was added as before. In order to obtain the initial values, a drop of sodium hydroxide solution was added to a known volume of the enzyme solution, which was then mixed with the sugar solution in the same proportion as in the experiment.

In the preliminary experiment the raffinose used was that supplied by Kahlbaum. The rotatory powers of the solutions were determined in sodium light.

The results obtained are exhibited in Table II, the values given under K being those deduced with the aid of the formula $K = \frac{10^5}{t} \log_{10} \frac{a}{a-x}$.

Table II.

Time.	Cane-sugar.			Raffinose (commercial).		
	34·2 grammes of cane-sugar (1/10 mol. $C_{12}H_{22}O_{11}$) + 4 c.c. of strong invertase extract per 1000 c.c.			59·4 grammes of raffinose (1/10 mol. $C_{18}H_{32}O_{16}.5H_2O$) + 4 c.c. of strong invertase extract per 1000 c.c.		
	α_D.	Per cent. hydrolysed.	K.	α_D.	Per cent. hydrolysed.	K.
mins.						
0	4·36	0·0	—	12·25	0·0	—
5	3·88	8·3	753	12·15	1·8	157
15	2·87	25·9	868	11·92	5·9	176
25	2·09	39·5	865	11·51	13·4	249
40	0·78	62·4	1062	11·14	20·1	243
60	−0·13	78·2	1102	10·63	29·3	251
95	−0·87	91·1	1106	9·95	41·6	246
140	−1·02	93·7	859	9·30	53·4	237
200	−1·10	95·1	656	8·57	66·6	238
260	−1·15	96·0	537	7·95	77·9	252
∞	−1·38	100·0	—	6·73	100·0	—

On plotting curves to represent the rates at which the two sugars are changed, it is seen that whilst one-half of the cane-sugar is hydrolysed in 33 minutes, the half of the raffinose is hydrolysed only after the lapse of

Table III.

Time.	Cane-sugar.			Raffinose (recrystallised).		
	34·2 grammes of cane-sugar (1/10 mol. $C_{12}H_{22}O_{11}$) + 4 c.c. of strong invertase extract per 1000 c.c.			59·4 grammes of raffinose (1/10 mol. $C_{18}H_{32}O_{16}.5H_2O$) + 4 c.c. of strong invertase extract per 1000 c.c.		
	α_{Hg}.	Per cent. hydrolysed.	K.	α_{Hg}.	Per cent. hydrolysed.	K.
mins.						
0	4·504	0·0	—	13·768	0·0	—
10	3·332	17·4	829	13·462	4·6	203
20	2·100	35·6	957	13·168	9·0	204
30	1·074	50·9	1043	12·918	12·7	200
40	0·306	62·3	1058	12·668	16·4	195
60	−0·848	79·4	1143	—	—	—
80	−1·490	88·9	1193	11·814	29·2	187
120	−1·810	93·6	997	11·044	40·6	189
160	−1·982	96·2	888	10·398	50·3	190
200	−2·114	98·2	868	9·850	58·5	191
∞	−2·238	100·0	—	7·065	100·0	—

126 minutes; in other words, it takes about 3·8 times as long to convert one-half of the raffinose into melibiose and fructose as it does to change one-half of the cane-sugar into dextrose and fructose.

For the following experiments the raffinose was purified by dissolving it in hot water, filtering the hot solution, adding a large bulk of alcohol to the filtrate and allowing it to stand during several days. The results are given in Tables III and IV.

Table IV.

Time.	Cane-sugar.			Raffinose (recrystallised).		
	68·4 grammes of cane-sugar (1/5 mol. $C_{12}H_{22}O_{11}$) + 4 c.c. of strong invertase extract in 1000 c.c.			118·8 grammes of raffinose (1/5 mol. $C_{18}H_{32}O_{16}.5H_2O$) + 4 c.c. of strong invertase extract in 1000 c.c.		
	α_{Hg}.	Per cent. hydrolysed.	K.	α_{Hg}.	Per cent. hydrolysed.	K.
mins.						
0	9·886	0·0	—	28·462	0·0	—
10	8·818	7·7	349	28·156	2·4	106
20	7·548	16·9	402	27·796	5·1	117
30	6·298	25·9	434	27·405	8·3	126
40	5·198	33·8	449	26·972	11·7	136
55	—	—	—	26·354	16·6	144
60	2·958	50·1	503	—	—	—
70	1·895	57·7	535	25·743	21·4	150
80	0·985	64·3	560	25·293	25·0	156
90	0·262	69·6	574	24·908	28·0	159
100	−0·366	74·1	587	24·517	31·1	162
115	—	—	—	23·962	35·5	165
120	−1·528	82·5	631	—	—	—
130	−1·932	85·4	643	23·352	40·3	172
140	−2·266	87·8	653	23·001	43·1	175
150	−2·562	89·9	666	22·667	45·7	177
160	−2·793	91·6	674	22·346	48·2	179
175	—	—	—	21·895	51·8	181
180	−3·172	94·4	694	—	—	—
190	−3·302	95·3	699	21·458	55·2	184
200	−3·446	96·3	719	21·166	57·5	186
220	−3·524	96·9	687	20·662	61·5	188
∝	−3·950	100·0	—	15·787	100·0	—

On plotting curves representing the rates of hydrolysis in the case of the solution of one-tenth molecular strength, it appears that whilst 50 per cent. of the cane-sugar is hydrolysed in 29 minutes, 50 per cent. of the raffinose is hydrolysed only after 158 minutes, the ratio being 1 : 5·4 as compared with the ratio 1 : 3·8 in the experiment with the commercial raffinose. Apparently, the recrystallised raffinose is less quickly hydrolysed than the crude material. In order to see if this were really the case, comparative experiments wer carried out, side by side, with the two kinds of raffinose and with cane-sugar; moreover, with the object of ascertaining the effect of further purification on the stability of the sugar in presence of the enzyme, the recrystallised raffinose was dissolved in "conductivity" water, the solution was mixed with alcohol and a stream of carbon dioxide passed through it during 30 minutes, this artifice having been found to be of service in purifying galactose; the solution was then filtered and a large bulk of alcohol added to the filtrate. The material obtained by this method is the "Raffinose III" in the following table. It was again recrystallised from a mixture of alcohol and "conductivity" water; the material thus obtained is marked "Raffinose IV." Equimolecular proportions of these samples of raffinose having been dissolved, the liquids were boiled to drive off any alcohol present, and were then diluted to the proper volume. Equal volumes of these solutions were measured out and to each was added the same number of cubic centimetres of a dilute invertase solution. The results are recorded in Table V.

Table V.

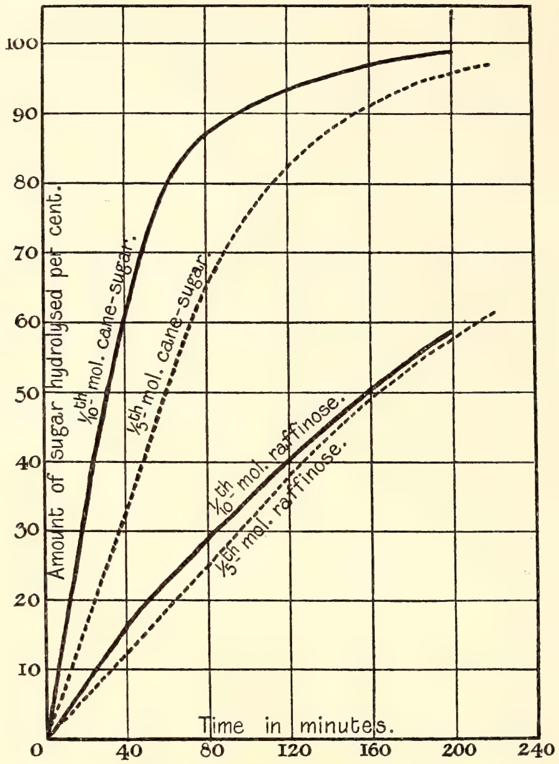
	Amount hydrolysed at 25°.			
	1 hour.	2 hours.	3 hours.	4 hours.
	Per cent.	Per cent.	Per cent.	Per cent.
Cane-sugar	96·2	—	—	—
Kahlbaum's raffinose	42·7	65·8	80·3	88·8
Recrystallised raffinose	38·1	61·9	76·4	82·8
Raffinose III	38·4	60·8	76·9	82·9
Raffinose IV	37·4	59·2	76·2	84·5

The various samples of raffinose used in this last experiment had the following specific rotatory powers:—

	$[\alpha]^{25°}_{Hg}$.
Kahlbaum's raffinose	122·5°
Ditto recrystallised	122·4
Raffinose III	121·6
Raffinose IV	122·2

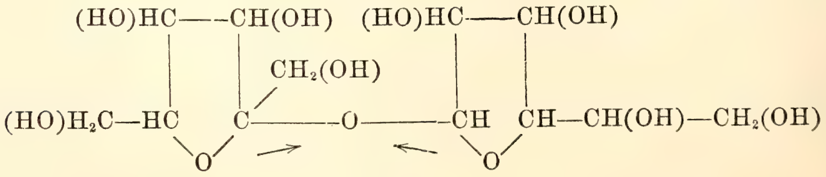
The low value obtained in the case of the third sample may be attributed to imperfect drying rather than to impurity. Probably, the greater activity of the invertase in the case of commercial raffinose was conditioned by the presence of traces of "acid" impurity (*cp.* X, p. 362).

The results afford clear proof that invertase is a far less effective hydrolyst of the cane-sugar section of raffinose than of cane-sugar itself, being at least five times as active towards the latter. This is most clearly exemplified in the following diagram.



Two explanations of the difference seem possible: either that the weighting has had the effect of twisting the oxygen centre or centres from which the hydrolytic attack proceeds somewhat further away from the junction at which the breakdown takes place on hydrolysis; or that the galactose interposes what is commonly known as steric hindrance, more or less blocking the way, as it were, to the hydrolytic agent. Unfortunately, we have no certain knowledge at present of the structure even of the biose cane-sugar, and that of derivatives such as raffinose is altogether problematic.

Cane-sugar is so different from other bioses with which it is isomeric that its constitution must be peculiar. The most probable structural and functional formula which can be devised at present is that proposed by Emil Fischer, when written in the following manner:—



The two arrows are introduced to indicate the direction from which hydrolytic attack may be supposed to proceed: a molecule open to attack in such manner would, doubtless, be far less stable than one in which only a single contiguous oxygen centre serves to direct the attack.

The hydrolysis of melibiose and of raffinose by emulsin lactase will be considered in a later communication; the hydrolysis of melibiose by acids and by melibiase will also be dealt with separately.

Studies on Enzyme Action. XII.—*The Enzymes of Emulsin.*

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Authors' title slip :—D. Q.

Subject slips :—

D 1850	Amygdalin, hydrolysis by emulsin to amygdonitrileglucoside.
D 8014	Emulsin (almond)—presence in, of three enzymes.
Q 1240	Amygdalase, presence of, in almond emulsin.
D 8012 Q 1230	Lactose, hydrolysis of, by glucolactase.
D 6300	Hydrogen cyanide (from amygdalin).
D 6350	Glucose (from amygdalin)].

Emulsin has frequently been the subject of discussion in this series of studies: thus the rate at which it hydrolyses milk-sugar was considered in No. II (vol. 73, pp. 507, 515) and its action contrasted with that of Kephir-lactase, which was shown to act more rapidly than emulsin. In No. III (vol. 73, p. 518) it was pointed out that whilst Kephir-lactase is controlled by galactose and scarcely at all by glucose, emulsin is controlled by glucose and only to a minor extent by galactose. These conclusions were confirmed by experiments with the methyl-glucosides and galactosides (p. 523). In No. V (vol. 74, p. 188) the question was discussed whether emulsin proper hydrolyses milk-sugar or whether, as Bourquelot and Hérissey have contended, emulsin contains a small proportion of lactase: against this assumption it was argued that the curve was not of the form to be expected if only a small quantity of lactase were present; that whereas Kephir-lactase was controlled by galactose alone, emulsin was most retarded by glucose and only to a slight extent by galactose, also that the curves for emulsin differed in character from those for lactase. Attention was directed, however, to Pottevin's statement that *Aspergillus niger* contains an enzyme capable of hydrolysing β-glucosides, but not β-galactosides nor milk-sugar. In No. X (B, vol. 79, p. 360) some of the statements made in earlier studies were rectified and it was announced that evidence had been obtained that the activity of ordinary emulsin is due, as the French workers had contended, to the presence of a distinct enzyme capable of hydrolysing β-galactosides, on which emulsin proper has no action, this enzyme affecting only β-glucosides.

While admitting this to be the case, however, we may point out that the conclusions we have to bring forward involve merely a different reading of the facts: the facts have been correctly advanced on both sides.

The explanation of the difference in the interpretations arrived at is simply that Kephir-lactase and emulsin-lactase are distinct enzymes—the one being a *galacto-lactase*, the other a *gluco-lactase*: the former being controlled by galactose, the latter by glucose. Probably, therefore, hydrolysis is effected in consequence of the attachment of the one enzyme to the glucose section and of the other to the galactose section of the biose.

In No. IX (B, vol. 79, p. 350) of these studies, proof was given by Messrs. Caldwell and Courtauld of the existence in yeast of a specific enzyme, *amygdalase*, capable of hydrolysing the bioside amygdalin into amygdonitrile-glucoside and a single molecule of glucose:

$$C_6H_5CH(CN)O{-}C_{12}H_{21}O_{10} + H_2O \rightarrow C_6H_5CH(CN)O{-}C_6H_{11}O_5 + C_6H_{12}O_6.$$

But amygdalin is resolved by emulsin into two molecules of glucose and one of the benzylidenecyanhydrol; this latter being unstable in presence of water decomposes into hydrogen cyanide and benzaldehyde. If both junctions were severed by one and the same enzyme, the effect produced would be one entirely *sui generis*, so far as our knowledge extends. From this point of view, the behaviour of emulsin is of special importance and has engaged our attention during several years past.

By systematically studying the action of acids on amygdalin, Messrs. Caldwell and Courtauld were led to discover that the two glucose sections of the molecule are more readily separated from one another than is the benzylidenecyanhydrol; they succeeded in verifying this conclusion by isolating Fischer's glucoside—as the compound of the cyanhydrol with glucose is termed—from the product obtained on partially hydrolysing amygdalin.

We have endeavoured to follow the course of change under the influence of emulsin. The analytical difficulties experienced at first were very serious, and trustworthy results were obtained only after long study of the methods.

Eventually, however, we succeeded in determining both hydrogen cyanide and glucose in a fairly satisfactory manner, although not with the degree of accuracy we desired.

The results of such determinations having shown that apparently the production of glucose was in advance of the production of hydrogen cyanide to a not inconsiderable extent during the earlier part of the change, it appeared probable that the action of emulsin was comparable with that of acids and that Fischer's glucoside was the main initial product of change: we therefore endeavoured to isolate this substance when the action was about half complete.

The neutral solution was carefully evaporated on the water bath and the syrup ultimately obtained was then extracted with ethylic acetate in the manner described by Caldwell and Courtauld.* The ultimate product was identical in crystallographic character, melting point and solubility with Fischer's glucoside and the melting point of a mixture of the two substances was that of the glucoside; it also gave an acetate indistinguishable from that prepared from Fischer's glucoside.

There can, therefore, be practically no doubt that besides *gluco-lactase,* emulsin contains a *β-glucase* capable of hydrolysing substances such as β-methylglucoside, together with a third enzyme, *amygdalase.* That gluco-lactase is present in addition to amygdalase is proved by the fact that the former may be destroyed by heating, whilst the latter persists.

In view of this result, we are justified in laying emphasis on the statement made in No. X of these studies:—"As the investigation is extended, the evidence becomes more and more convincing that the action which an enzyme exercises is specific; in other words, that it is limited to compounds of a particular type—to a greater extent, indeed, than is recognised in earlier communications of this series." In fact, it is difficult to overrate the value of enzymes as diagnostic agents.

In No. VII (B, vol. 76, p. 592) of these studies, dealing with the synthetic action of enzymes, evidence was adduced tending to prove that emulsin and maltase give rise respectively to maltose and "isomaltose." In view of the results now brought forward, it is clear that the nature of the synthetic processes must be regarded as uncertain until definite proof is obtained that this or that enzyme is the one concerned in the formation of a particular biose. At present we see no reason, however, to doubt the validity of the arguments previously adduced, the results obtained on repeating the experiments having uniformly served to confirm the early observations.

But in extending the inquiry painful experience has convinced us that it is full of pitfalls and difficulties: it was always obvious that the enzymes were mixtures, and the evidence constantly forced upon us that the products are mixtures has been in no sense a surprise. To carry the investigation to a successful issue, we have not only to overcome the difficulties incident on the preparation of considerable quantities of the enzymes in suitable condition, but also to devise special methods of separating, purifying and definitely characterising the products.

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* 'Chem. Soc. Trans.,' 1907, vol. 91, p. 670.

Preparation of Emulsin.—Emulsin being an article of commerce, a dried solid preparation obtained from Merck, of Darmstadt, was made use of in the experiments referred to in the earlier communications of this series. This was always found to be active towards both amygdalin and milk-sugar. A similar preparation obtained from Kahlbaum in 1906 was almost worthless, being without action on milk-sugar even at 36° and acting only slowly on β-methylglucoside and salicin. We were led by the observations of these differences to prepare fresh emulsin from almonds, in the expectation that it would be much more active than a dried preparation; this proved to be the case.

The almonds—either the sweet or bitter variety may be used—were reduced to a pulp by passing them between rollers; the pulp was then pressed to remove as much as possible of the oil and afterwards macerated during about 24—48 hours with about two or three times its weight of water containing a little toluene. The most active preparations are obtained by extracting at a moderately low temperature, about 10°—20°. The cloudy liquid obtained on filtering the extract was mixed with two drops of acetic acid per 100 c.c., to precipitate part of the albuminoids present; the relatively clear liquid obtained, after separating the precipitate by filtration, was then mixed by degrees with about an equal volume of alcohol. The precipitate thrown down by the alcohol was allowed to subside and then washed by decantation with alcohol; finally, it was removed to a filter-paper. The adhering alcohol having been absorbed as far as possible by means of filter-paper, the moist solid was shaken up with distilled water containing toluene, the amount of water being varied according to the strength of the extract desired. After remaining in contact with the solid during 48 hours, at 37°, the solution was filtered and the residue again extracted with water. The extract thus obtained is a colourless, clear liquid, admirably suited for polarimetric work.

Lactoclastic Power of "Emulsin."—In comparative experiments made with such a solution, at 15° and 36°, with milk-sugar and β-methylglucoside, the glucoside underwent hydrolysis to a considerable extent at the lower temperature, whereas the milk-sugar was but slightly attacked; at the higher temperature, both were hydrolysed comparatively rapidly.

On heating the extract at 45° during three hours it was entirely deprived of the power of hydrolysing milk-sugar; it retained its activity as a hydrolyst of β-methylglucoside, amygdalin and salicin, however, not only after 20 hours' heating at 45°, but even when heated during several hours at 55° in presence of these hydrolytes; the enzyme was practically destroyed by heating at about 59°.

The preparation obtained by macerating almonds with water at about 57° was found to be active towards the three glucosides at 38°, but when heated to 62° it became inactive.

By macerating almonds with water at about 0° and then again macerating the extracted paste with a further quantity of water at 45°, extracts were obtained from which "emulsins" were prepared in the manner described. Both preparations hydrolysed milk-sugar at 38°; only that made at the lower temperature, however, produced perceptible hydrolysis at 15°—an indication that the gluco-lactase had been preferentially extracted at the lower temperature.

Cane-sugar, maltose and β-methylglucoside were not in the least affected by these preparations.

A solution containing 5 per cent. of lactose was completely hydrolysed at 38° in the course of a week, when presumably the enzyme was in equilibrium with the products of change; a volume of the sugar solution equal to that first taken was then added: it was found that hydrolysis took place as before and became complete, showing that the enzyme had retained its activity. Extracts prepared in the manner described, if maintained sterile by means of toluene, have been found to retain their activity almost unchanged during many months.

A typical series of values obtained with emulsin, prepared by extracting almond paste at about 0°, is contained in Table I.

Whereas, in the experiments recorded in No. II of these studies, made with Merck's relatively inactive emulsin, a series of decreasing values were obtained, the values recorded in the upper part of Table I form an increasing series. The change follows a straight-line law during at least seven hours until about 30 per cent. of the sugar is hydrolysed. Viewed in the light of the explanation formerly given, which has found general acceptance,* the results are easily explained and significant: obviously only a very small quantity of a highly active enzyme was present—so much so, indeed, that the magnitude of the active system remained constant during a considerable period, until as much as 30 per cent. of the hydrolyte had been attacked.

Additional proof that this explanation is admissible is given in the lower

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* Compare Bayliss, 'Archives des Sciences Biologiques,' 1904, vol. 11, suppl., p. 261.

Table I.

5 grammes milk-sugar per 100 c.c. X = percentage amount hydrolysed.

Time.	10 c.c. enzyme.		20 c.c. enzyme.	
	X.	K.	X.	K.
hours.				
2	4·4	0·00975	8·3	0·0018
3	6·8	0·0102	12·1	0·0184
5	13·2	0·0113	21·4	0·0209
7	18·0	0·0123	31·9	0·0238
10	20·8	0·0101	42·3	0·0239
24	40·5	0·0094	61·0	0·0170
27	42·5	0·0089	64·5	0·0166
75	70·3	0·0070	83·3	0·0104

Time.	40 c.c. enzyme.		60 c.c. enzyme.	
	X.	K.	X.	K.
hours.				
2	15·2	0·0358	18·5	0·0444
3	18·5	0·0296	22·7	0·0373
5	21·1	0·0251	36·5	0·0394
7	33·5	0·0253	44·9	0·0380
9	42·2	0·0264	52·5	0·0360
10	44·0	0·0252	53·5	0·0332
24	73·4	0·0240	76·0	0·0258

part of the table, in which are recorded the results of experiments made with four and six times the amount of enzyme used in the experiment just described. It will be noticed that when the fourfold amount was used, the quantity of enzyme was such that the hydrolyst no longer had unlimited choice, but was forced to compete for the sugar molecules: being dependent on the proportion of unhydrolysed molecules, the rate of change followed the simple mass-action law, as shown by the approximately constant value of K after about three hours.

Using the fourfold and sixfold amount of enzyme, the rate of change is such that the influence of the products of change soon becomes of consequence; a decreasing series of values is therefore obtained. On comparing the values found, it is obvious that at first, when the amount of enzyme used was doubled, the rate of change was doubled; subsequent additions, however, did not produce the proportionate increase in the rate of change.*

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* The experiments were all carried out at 38° in precisely the manner already explained (No. II). Jena-glass vessels were used throughout, with the exception of the pipettes. The data given are quoted as illustrations of the results obtained in a number of similar experiments.

Amount of enzyme.	Velocity constant K.
10 c.c.	0·0107
20	0·0212
40	0·0279
60	0·0385

While the evidence secured is sufficient, we consider, to prove the existence of a lactase in almonds distinct from emulsin proper (β-glucase), it in no way serves to distinguish the enzyme from that in Kephir grains, the action of which was considered in Nos. II and III. The proof on which we rely as a means of differentiating the two enzymes is that the one is controlled by glucose, the other by galactose. The effect of these hexoses on Kephir-lactase was considered in No. III. Results are now recorded in Table II which illustrate the behaviour of emulsin-lactase prepared in the manner described above; they afford complete confirmation of those obtained four years ago, which were then properly interpreted as proof of the non-identity of the two enzymes, although it was not then realised that emulsin proper (β-glucase) was incapable of hydrolysing milk-sugar. Such evidence, in our opinion, is a clear indication that the hydrolysis of milk-sugar is consequent on the attachment of the enzyme in the one case to the galactose section and in the other to the glucose section of the biose; we may point to this conclusion as a striking illustration of the diagnostic value of what may be termed the *control method* which has been developed in the course of these studies and as being also an interesting extension of our conception of the manner in which enzymes may affect hydrolysis.

The Amygdaloclastic Activity of Emulsin.—Although the individual substances which are formed when amygdalin is hydrolysed—hydrogen cyanide, benzaldehyde and glucose—may be estimated more or less easily when apart, serious errors arise when the ordinary analytical methods are applied to their determination in admixture.

Ripper's method* of determining benzaldehyde gives low results, even with benzaldehyde alone, the values varying with the dilution. It is altogether vitiated by the presence of hydrogen cyanide. All attempts we have made to fix the cyanide as an insoluble or non-volatile cyanide have been unsuccessful, some always passing over during the removal of the benzaldehyde by distillation.

The determination of benzaldehyde in the form of a difficultly soluble hydrazone is unsatisfactory, chiefly on account of the difficulty of securing its conversion completely into hydrazone and because of the readiness with which it is oxidised.

Liebig's method of estimating hydrogen cyanide cannot, as a rule, be applied directly to the solution in which hydrolysis has taken place, as the emulsin is liable to interfere with the determination of the end point, by rendering the solution turbid. The method

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* 'Zeit. anal. Chem.,' 1902, vol. 41, p. 61.

Table II.

5 grammes milk-sugar per 100 c.c. 40 c.c. enzyme extract* + 5 grammes glucose or galactose.

Time.	Amount hydrolysed.		
	No addition.	Glucose.	Galactose.†
hours.	grammes.	gramme.	grammes.
2	0·35	0·05	0·36
5	0·85	0·10	0·88
10	1·225	0·37	1·23
24	2·025	0·75	1·96

Time.	Amount hydrolysed.		
	No addition.	No. 1 galactose.	No. 2 galactose.
hours.	grammes.	grammes.	grammes.
19‡	1·59	1·58	1·60
21	1·71	1·68	1·69
27	1·86	1·83	1·87

5 grammes milk-sugar per 100 c.c. 30 c.c. enzyme extract§ + 3 grammes glucose.

Time.	Amount hydrolysed.	
	No addition.	Glucose.
hours.	grammes.	gramme.
2	0·169	0·095
5	0·412	0·175
7	0·530	0·220
10	0·730	0·280
24	1·120	0·572

* From bitter almonds.

† Purified material.

‡ These results illustrate the character of the action during the later stages of hydrolysis, those above during the earlier; the two samples of galactose were from different sources: both were purified by careful recrystallisation. The two sets of observations were made at intervals three months apart.

§ From sweet almonds.

which was adopted by Tamman,* involving the precipitation of the cyanide by excess of silver nitrate and the subsequent determination of the excess, has frequently given impossible results in our hands: apparently the benzaldehyde interferes.

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* 'Zeit. physiol. Chem.,' 1892, vol. 16, p. 286.

Ultimately we were led to devise a method which not only renders possible the estimation of hydrogen cyanide with a fair approximation to accuracy, but also permits of a considerable number of determinations being taken in hand in rapid succession—a matter of some importance. The solution (10 c.c.) in which hydrolysis has taken place is added to a decinormal solution of silver nitrate (20 c.c.) previously mixed with an equal bulk of a decinormal solution of sodium acetate. The mixture is then heated (5—10 mins.) on a water bath. A bulky precipitate of silver cyanide is thus formed, which may be set aside until it is convenient to complete the analysis. The precipitate, having been filtered off and washed with boiling water, is transferred to a beaker with the aid of about 50 c.c. of water; it is then dissolved in 5 c.c. of strong ammonia, the precipitate adhering to the filter being removed by means of 5 c.c. of ammonia, diluted with 25 c.c. of water, and the beaker in which precipitation is effected also washed out with 5 c.c. ammonia and 30—40 c.c. of water. The combined solutions are mixed with 4 c.c. of a normal solution of sodium chloride and poured through a thistle funnel into a round-bottomed Jena flask containing a solution of 25 grammes of tartaric acid. The liquid is raised to the boiling-point and distilled during 15 mins. in a current of steam, the distillate being received in a flask containing a slight excess of potash. The solution is then titrated with a solution of silver nitrate. A small amount of benzoic acid often distils over, but this does not appear to interfere with the titration.

To determine the amount of glucose produced during hydrolysis, the standard method described by Brown, Morris and Millar,* involving the use of Fehling's solution, was adopted, as it was found that neither amygdalin nor hydrogen cyanide had any reducing action on Fehling's solution and that emulsin had a very slight action, the effect of which was determined and allowed for. The procedure is as follows:—

10 c.c. (or 20 c.c. at the end of the first hour) of the liquid in which the action has been stopped by adding a drop of sulphuric acid are introduced into a Wurtz flask and the sulphuric acid neutralised by adding a previously determined quantity of N/5 potash; the liquid is then diluted to about 125 c.c. and distilled by passing in steam during about 30 minutes. The liquid is next rinsed into an evaporating basin, evaporated to a small bulk, transferred to a 50-c.c. flask, cooled and diluted to 50 c.c. The required quantities (25 c.c.) of each of the two Fehling's solutions are mixed in a 400-c.c. wide Jena glass beaker with such a quantity of water that addition of the sugar solution will make the volume up to 100 c.c.; this beaker is heated in a boiling-water bath during 5 mins., then removed and 25, 15 or 10 c.c. (according to its strength) of the solution in which glucose is to be determined is added. The beaker is then replaced in the water bath, covered with a clock-glass and heated during exactly 12 mins. The precipitated cuprous oxide is collected on an asbestos filter in a Soxhlet tube, washed with about 200 c.c. of boiling water, then with 10 c.c. of alcohol and dried in a water oven. The oxide is oxidised by heating in a current of air and then reduced to copper by heating in a current of purified dry hydrogen, cooled and weighed. The initial oxidation is necessary, in order to prevent volatilisation of part of the copper, which occurs when the cuprous oxide is heated directly in a current of hydrogen, probably owing to the presence of organic matter.

Using Fischer's glucoside as test material, which is resolved simply into glucose, benzaldehyde and hydrogen cyanide by emulsin, the amounts of hydrogen cyanide found have been uniformly low—on the average from one to two units below the calculated percentage values—so that the process is certainly open to improvement and simplification; we hope to improve it.

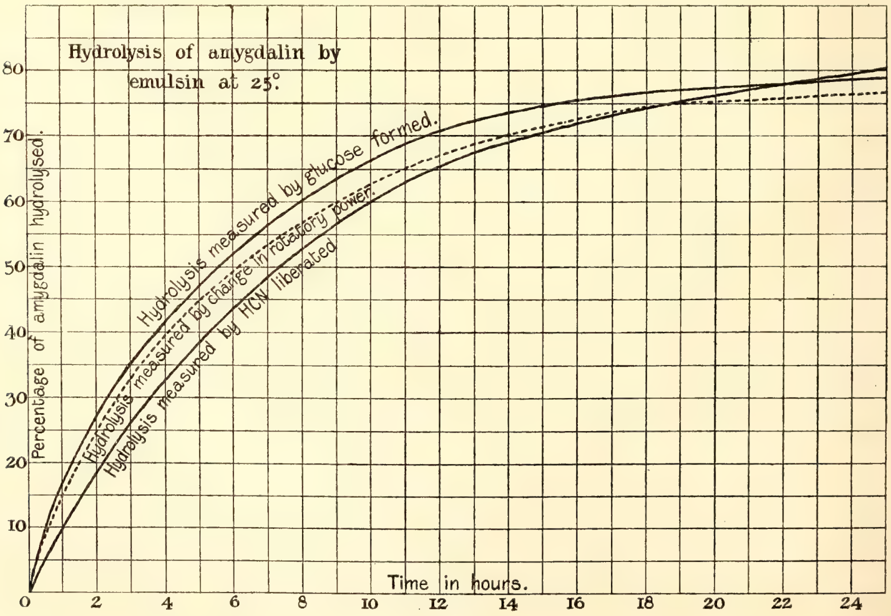
In numerous experiments in which the proportions of reducing sugar and hydrogen cyanide formed on digesting amygdalin with emulsin have been determined, the extent to which hydrolysis takes place, as measured by means of the cyanide, has always been found lower than that measured by the amount of sugar produced—assuming this to be glucose—to an extent considerably beyond the probable error affecting the determinations. The following series of percentage values may be quoted in illustration:—

---

* 'Chem. Soc. Trans.,' 1897, vol. 71, p. 281.

Time.	Rotatory power.	Hydrogen cyanide.	Glucose.
hours.			
1	14·6	9·9	16·7
3	33·0	26·7	35·5
5	44·7	38·6	46·1
7	53·7	48·5	56·7
9	59·9	56·9	63·9
11	65·5	63·3	69·0
25	76·8	80·7	79·1

The experiment is one of the few in which it was found to be possible to determine the hydrogen cyanide by direct titration. It will be seen that the difference increases as the action proceeds, and then diminishes; the change indicated by the variation in rotatory power also rises to a maximum, and then diminishes. The relationship is more obvious in the following diagram.



The following values were obtained in an experiment in which the precipitation method was used in determining the cyanide:—

Time.	Glucose.	HCN.
hours.		
2	38·3	31·0
3	48·0	41·0
4	54·4	49·5
5	60·8	55·75
6	65·6	61·25
24	94·5	93·0

An emulsin which had been heated during three hours at 55°, although weaker, produced a smaller proportion of cyanide as compared with that of glucose than the unheated extract—

Time.	Glucose.	HCN.
hours.		
17·5	77·6	68·8
40·5	89·0	74·5

The amount of Fischer's glucoside we have separated from partially hydrolysed material has been small—at most 3 or 4 per cent. of the weight of the amygdalin used; apparently there is no great difference between the rates at which this glucoside and amygdalin are hydrolysed by the β-glucase. In some cases we have even failed to isolate a crystalline product and have had reason to suspect that the glucoside has been in part converted during its manipulation into the stereoisomeride.

A Further Note on the Nutrition of the Early Embryo: with Special Reference to the Chick.

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(Communicated by Professor C. S. Sherrington, F.R.S. Received April 3,—Read May 21, 1908.)

In furtherance of the views put forward regarding the nutrition of the early embryo by the author in a preliminary note read before the Royal Society in February, 1905, the following series of experiments was carried out.

The changes that take place between the growing embryo and the maternal secretion are, in the mammalia, not easy to study, on account of the difficulties to be encountered, such as the minute size of the embryo, and the small amount of uterine secretion available. In birds, on the other hand, the uterine secretion, viz., the white of the egg, is abundant. The growing embryo can be examined easily at any stage which may be desired, and, since all the changes which take place do so within the limits of the shell, the products of these changes are capable of accurate analysis.

It appeared to the writer, as will be indicated by a reference to the note referred to, that the uterine secretion was assimilated by the growing embryo not by a process of osmosis, but by direct digestive changes brought about by the outermost cells of the embryonic vesicle. Such digestive changes, if they did occur, would naturally lead to the presence of digestive products in the uterine secretion in contact with the growing embryo, if not to an actually demonstrable presence of an enzyme in this secretion.

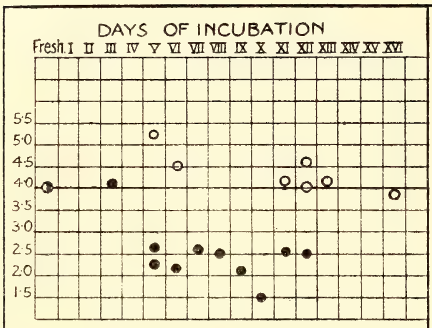
In the note also it was surmised that the uterine secretion of mammalia contained albumin and mucin. The examination of the resting uterus of a cow established the presence of proteid, mucin, and salts, as did also the examination of the uteri, some resting, some in the pre-œstral stage, of seven bitches. The examination of the uterus of a cow in the pre-œstral stage was interesting: externally both horns were injected, one horn, much larger than the other, was surmised to be the horn of a previous gestation; internally the lumina were bathed in a watery secretion tinged, especially near the Fallopian tubes, with blood. It was noticeable how watery the secretion was in comparison with the somewhat mucoid condition of the same secretion in the resting stage. The cervical and vaginal secretions, on the other hand, were profuse and extremely mucoid. The above secretions, both uterine, cervical, and vaginal, were slightly alkaline to litmus.

A series of experiments was made upon the egg-white surrounding the developing chick. It is well known how this substance decreases in bulk as development proceeds, but apparently no means have been taken to show what changes are undergone during this process. In egg-white one has to deal with three main distinct components—the proteid, the water, and the salts. Milroy states that normal egg-white contains 10 to 13 per cent. of proteid (*i.e.*, approximately 87 to 90 per cent. of water), but his results were obtained after straining through muslin, which naturally would lead to a higher percentage of water when compared with results obtained where no straining was introduced.

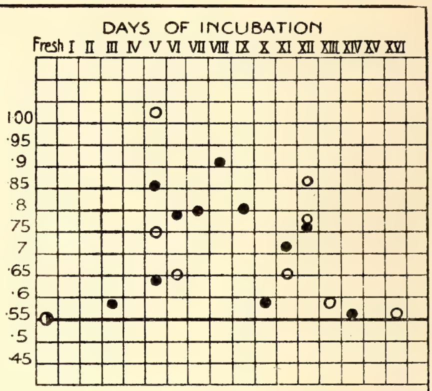
As incubation proceeds, the most obvious change in the egg-white, after its diminution in bulk, is its extreme tenacity, so much so that it is with great difficulty that it can be removed for examination. It also becomes more yellowish-green in colour, probably due to its more concentrated state.

It is evident, therefore, that the loss of water has been disproportionate to the loss of proteid. That this is so is demonstrated by the percentages of water taken at different stages, when it will be seen that the disproportion increases as incubation proceeds (*vide* Diagram).

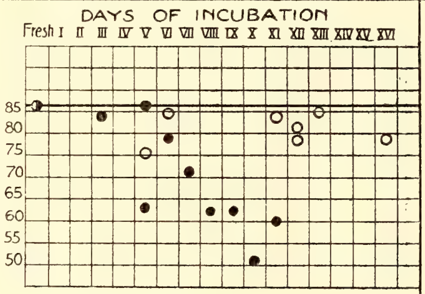
The most remarkable change is that which affects the reactions of the substance: instead of being soluble in distilled water, as is fresh egg-white, a white coagulum is formed at the interface between the water and the proteid, rendering solution difficult; if, however, the substance is shaken up with distilled water in a flask over a period of some days, with a few drops of xylol added in order to ensure sterility, it will be found that on each day a certain portion of the substance will have become soluble, leaving a gelatinous mass in which, on section, fœtal villi can be demonstrated. In the early stages it is somewhat soluble in normal saline solution, but later it becomes more and more difficult to dissolve. On heating, the substance becomes dried, but fails to coagulate. It is precipitated by dialysis. At this point certain changes are going on in the salts of the egg-white, as demonstrated by the amount of ash after incineration. It will be seen (*vide* Diagram) that while in relation to the amount of egg-white the percentage of water is rapidly decreasing, that of the salts remains much the same for unfertilised as for fertilised eggs (whether due to osmotic influence or not is conjecturable). But it is evident that as the egg-white loses its water the proteid becomes more and more concentrated, so that, taken in relation to the amount of proteid (dried at 110°), that of the salts shows a more or less corresponding decrease, which is interesting from a comparative tabulative point of view. Since the percentage of salts, however, to the amount of egg-white is much the same for both conditions, it seems that one cannot ascribe the changes in reaction of the egg-white in the fertilised egg as being due to either a diminution of, or an excess in, the percentage of salts present. This has a bearing upon the influence of salts in the building up of certain proteids. It also suggests the inference that the water of the egg-white is removed at a much greater rate, for the purposes of the embryo's nutrition, than either the salts or the proteid.



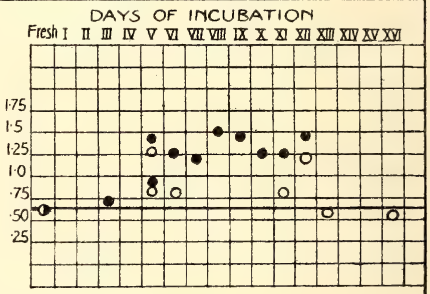
The % of Ash to Dried Proteid
○ *Unfertilized Eggs.*
● *Fertilized Eggs.*



The % of Ash to Egg-white
○ *Unfertilized Eggs.*
● *Fertilized Eggs.*



The % of Water
○ *Unfertilized Eggs.*
● *Fertilized Eggs.*



The % of Ash to Water
○ *Unfertilized Eggs.*
● *Fertilized Eggs.*

What the composition of the proteid of the egg-white in the fertilised egg is is not clear. Attempts to analyse it show it to be globulin, though by no means partaking of all the reactions of this class of substance.

Experiments were conducted to ascertain the presence or absence of albumoses, peptones, etc., in the egg-white of developing eggs. The methods adopted were: (1) To shake up the insoluble white with distilled water; through the coagulated interface the albumoses and peptones passed into solution and gave a well-marked biuret reaction. (2) To add 10 times its bulk of absolute alcohol to the egg-albumin, to stir thoroughly, allowing the alcohol to remain for at least seven days. At the end of that time all the albumins and globulins were coagulated and the albumoses and peptones precipitated. On washing the filtered mass with distilled water, the latter were rendered soluble and passed out into the filtrate, where they were examined. From the sixth day onwards distinct evidence was obtained by means of the biuret reaction of the presence of albumoses and peptones. Examination of the yolk and of the amniotic fluid by the same means failed to elicit their presence in these substances.

Occasionally, it may be remarked, the biuret gave a reduction on standing, also obtained on boiling. The phenomenon was as follows:—The rose pink appeared readily, to quickly become a brown solution; on standing, the brown was precipitated in the form of a flocculent precipitate, the pink colour returning to its original shade when the precipitate had settled (at the end of 18 hours). What this reducing agent is is not known, it is improbable that it is a form of sugar.

J. Starke has carried out an experiment which may be referred to here. He found that if a greatly diluted albumin solution, any pre-existing globulin having been removed by filtration, is kept at 56° C. or over for some little time, it contains a proteid with the following reactions:—(1) It is precipitated by CO_2, acids, and the addition of a very small quantity of alkaline-earth salts. (2) Precipitated by dialysis. (3) Can be salted out by $MgSO_4.NaCl.KCl$. (4) Soluble in dilute alkalies and very dilute acids with absorption of the same. (5) Insoluble in H_2O and dilute neutral salts (it dissolves in the latter only if a trace of alkali is present). (6) It holds less ash than albumin. (7) It contains less sulphur than albumin. (8) It contains more water than coagulable proteid. (9) It is not heat coagulable. (10) It forms an opalescent solution like all globulins. (11) It sets free more reducing substance on boiling with acid than albumin. (12) It is sensitive to the influence of salts in an acid solution, not in an alkaline. (13) The neutral salts play, under the same circumstances, a *rôle* such as they do with globulins.

Examination of unfertilised eggs which have been incubated along with the fertilised shows that no material change has taken place in the reactions of the egg-white of the former, and so heat alone cannot be said to be the cause of the changes noticed in incubating developing eggs. Again, whilst this substance is admittedly more watery than coagulable proteid (*e.g.*, egg-white), our substance is definitely more concentrated than fresh egg-white. Both, however, are precipitated by dialysis. Both can be salted out by $MgSO_4$. Both are insoluble in water. Both are uncoagulated by heat. Both form opalescent solutions. It is questionable whether our substance holds less ash than albumin, it is certainly not readily soluble in dilute alkalies and very dilute acids. So that, although both substances differ from albumin in many similar particulars, they cannot by any means be said to be identical. On the whole it is sufficient to say that we are dealing with a substance of a proteid nature, bearing a resemblance to the group known as globulins. Until some new methods of proteid classification are introduced it is impossible to be more definite in one's description.

L. Moll has found that by warming albumin to 60° C., in the presence of an equal quantity of 0·079-per-cent. Na_2CO_2, it is converted into a globulin which appears to be similar to natural globulin, the amount of globulin being dependent on the concentration of the albumin solution. The carbonates, bicarbonates, and phosphates of the alkaline metals work equally, but weaker than the hydrates. While, according to Tarchanoff and Zoth, if hen's eggs are placed for two to three days in 10-per-cent. KOH, then the egg-white is no longer coagulable in the usual way on being boiled, but sets into a vitreous transparent jelly, as the salts are diminished while the alkalies are increased in amount. In both experiments are seen the effects of alkalies on egg-white, whereby this substance is apparently altered in a somewhat similar fashion to that produced by the action of the developing chick; unfortunately, too little is said of the reactions of the substance produced in these experiments to justify any definite comparison.

It is not necessary to enter into a minute description of the embryology of the chick; suffice it to say that the action of the chick on the white takes place by means of the chorionic epithelium, the products up to the fourth day being conveyed directly to the embryo by the mesoblast. When, however, the mesoblast splits to form the cœlom, the connection between the embryo and the chorion is threatened, but about the fourth day the allantoic stalk reaches the chorion, and thus bridges the gap, allowing the process to continue without interruption. Finally, the allantois is said to envelop the remaining egg-white so as to accelerate its assimilation, which is completed by the end of incubation. It thus follows that, in addition to its function as the respiratory organ, the allantois serves the purpose of conveying the nutrition of the egg-white to the growing embryo.

The experiments made upon the developing chick recorded above have been repeated during this year (1907), and have fully confirmed the results obtained in 1905 and 1906.

My very best thanks are due to Professor B. Moore and Dr. H. E. Roaf for their kind assistance.

Conclusions.

1. The secretion of the resting mammalian uterus contains protein, mucin, and salts; during the pre-œstral stage the proportion of mucin is decreased.
2. The profuse mucinous secretion of mammalia during pre-œstrus is derived not from the body of the uterus, but from the cervix and vagina.
3. The nutrition of the embryonic chick is not dependent upon the yolk alone, but also upon the egg-white.
4. Assimilation of the egg-white is divisible into three heads—the water, the salts, and the proteid.
5. Of the three, the water is at first extracted at the most rapid rate, *i.e.*, the percentage of water decreases as incubation proceeds.
6. The percentage of salts in the egg-white remains more or less unchanged throughout incubation.
7. The proteid of the egg-white is assimilated not by a process of osmosis, but by a process of digestion performed by the chorionic cells.
8. During this process the egg-white is considerably altered in composition and reaction, being converted, as incubation proceeds, into a more and more vitreous mass with a peculiar set of reactions, the outstanding reaction being that demonstrating the presence of albumoses and peptones.

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On Reciprocal Innervation in Vaso-motor Reflexes and the Action of Strychnine and of Chloroform thereon.

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(Received January 20,—Read March 12, 1908.)

(From the Physiological Laboratory of University College, London.)

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I. INTRODUCTORY.

In a paper published in the year 1893, after describing various experimental results on vascular reflexes, I made use of the following words: "the simplest explanation of the above results is the hypothesis that the vaso-motor centre consists of a constrictor and a dilator part, the depressor nerve acting in an inhibitory manner on the former, and in an exciting manner on the latter, while pressor nerves act in an opposite way on both."*

The evidence brought forward at that time in support of this view was mainly indirect, and in no way comparable with the brilliant experimental proofs afforded by Sherrington's masterly researches on the similar phenomena in the case of the reflexes to voluntary muscles. These latter results have, however, given renewed interest to the question, and the following pages contain an investigation whose object was to obtain direct experimental evidence whether or not the phenomena of vascular reflexes can be brought into line with those affecting skeletal muscle.

Since the publication of my paper referred to, certain incidental observations have been brought forward at various times tending to confirm the above hypothesis. These will best be discussed under the separate headings of the next section.

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* "On the Physiology of the Depressor Nerve," 'Journ. of Physiology,' vol. 14, p. 317, 1893.

Before proceeding to the experimental results, it will be advisable to make clear the meanings attached to some of the words used. By "vaso-motor" I intend to include not only vaso-constrictor effects, as is sometimes done, but also those of a vaso-dilator nature. Both actions are, in fact, vaso-*motor*, one producing movement in the sense of narrowing of vessels, the other widening. Similarly, by vaso-motor centre, or centres, I mean the centres from which are given off both the vaso-dilator and vaso-constrictor fibres, whatever may be the situation of these centres.

It is also necessary to remember that under normal conditions the arterioles are in a state of moderate contraction or tone, which may continue even when these vessels are separated from connection with the central nervous system. This tone is probably a normal characteristic of smooth muscular tissue; but, apart from this, it is kept up by the contractile reaction of the arterial muscle to the distending force of the heart-beat,* as well as by the effect of the internal secretion of the adrenals, in the case of those arterioles supplied with sympathetic nerve-fibres.†

This inherent muscular tone of the arterioles can be affected in two directions by impulses from the central nervous system, at all events in the case of most organs of the body. It can be increased by impulses down vaso-constrictor fibres or diminished (inhibited) by impulses down vaso-dilator fibres. Moreover, these impulses may be continuous, resulting from a state of tonic excitation of their respective centres, in all probability of reflex origin.

It is then obvious that, when we observe a vaso-dilatation in a particular organ in response to excitation of an afferent nerve, this result may be due to two things, either an excitation of vaso-dilator fibres or, supposing that the vaso-constrictor centre is in a condition of tonic excitation, to an inhibition of this centre, resulting in a cessation of the vaso-constrictor impulses previously producing increased contraction of the arterial muscle. Both results may be simultaneously brought about.

Conversely, when what is conveniently called a "pressor" reflex is brought about, that is, one associated with increased contraction of arterioles and consequent rise of arterial blood-pressure, we may have both excitation of vaso-constrictor fibres and inhibition of tone in the vaso-dilator centre.

Now, in order to investigate experimentally the various possibilities referred to, it is necessary to be able to make use of organs which are supplied with both vaso-constrictor and vaso-dilator nerves, and these two kinds of fibres must pass to the organ in anatomically distinct nerve-trunks. When this is the case, it is possible to decide by section of one or the other whether a particular result is due to one of these alone. For instance, if we observe a vascular dilatation in the sub-maxillary gland in response to excitation of the central end of the depressor nerve, we can decide by section of the cervical sympathetic nerve whether the effect is still present. If so, it cannot be due to inhibition of constrictors alone, there must be also excitation of dilators in the Chorda tympani nerve.

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* W. M. Bayliss, 'Journ. of Physiology,' vol. 28, p. 220, 1902.

† T. R. Elliott, 'Journ. of Physiology,' vol. 32, p. 401, 1905.

There are, unfortunately, very few organs, accessible to investigation, which satisfy these conditions. Such are the sub-maxillary gland, the tongue, the external ear in the rabbit, the penis in the dog and the limbs. On all of these organs I have made observations.

General Methods.—All animals used were anæsthetised with A.C.E. mixture and morphine, except in the case of rabbits, where ether was used instead of A.C.E. mixture for reasons which will be seen later. With the exception of the submaxillary gland, all the organs investigated were placed in oncometers of appropriate form and the changes of volume recorded by a piston-recorder. In the plethysmographic experiments curare was usually found to be necessary. The arterial blood-pressure was always traced simultaneously with the plethysmographic curve.

II. Reciprocal Innervation in the Normal Animal.

There are, in the investigation of this question, four cases to be considered. In a reflex producing fall of blood-pressure with general dilatation of arterioles, is there evidence of both excitation of vaso-dilator nerves and inhibition of central vaso-constrictor tone? In a reflex of the opposite kind, is there evidence of both excitation of vaso-constrictor nerves and inhibition of central vaso-dilator tone?

1. *Depressor Reflexes.*

These are to be obtained, as is well known, by excitation of the central end of the depressor nerve in the rabbit, also from the central end of the vagus in the cat, and, under certain conditions, not exactly definable, from the central end of the vagus in the dog.

The discoverers of the depressor nerve, Ludwig and Cyon, apparently regarded its action as purely inhibitory on the vaso-constrictor centre.* This was also the usual opinion of subsequent workers until the publication of my paper above referred to. Ostroumoff,* however, in his work on the dilatation of cutaneous vessels, had already expressed the view that, in this particular case, the action of the depressor nerve is due to an excitation of vaso-dilator fibres and not to an inhibition of constrictor tone. Biedl,† at a later date, came to the same conclusion with regard to the vascular innervation of the adrenals. Cyon,‡ himself, on the other hand, strenuously opposes this interpretation of the facts. None of these observers recognises the possibility of both effects being present at the same time.

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* 'Ber. d. k. Sächs Ges.,' Math. phys. Classe, vol. 18, p. 318, 1866.

(i) *Excitation of Dilators.*—All the vaso-dilator nerves to the posterior limbs are contained in the dorsal roots of the sacral plexus.§ All the constrictors are situated in the abdominal sympathetic, having left the spinal cord in the white rami from the eleventh thoracic to the third lumbar segments inclusive.|| If, then, the spinal cord be transected at the fourth lumbar segment the hind-limbs will be, as regards their vaso-motor supply, in connection with the central nervous system by means of vaso-constrictor fibres only; and, if the abdominal sympathetic chain on both sides be extirpated, the dilators alone will be left. When this latter operation has been performed, it is found that dilatation of the limbs can still be obtained on exciting the depressor nerve. This can only be due to excitation of vaso-dilator fibres. The evidence for this statement is given in my paper above-mentioned. I will, therefore, merely refer to the experiment shown in fig. 10, p. 293, of that paper.¶

The objection may be made that the dilator impulses to the limbs are of a peculiar nature, being antidromic. It is, therefore, important to test the behaviour of organs, such as the submaxillary gland, the penis, or the tongue, where the dilators are of the recognised type.

The submaxillary gland suggests itself at once for this purpose, since it is easy to observe changes in the venous outflow by inserting a cannula in the peripheral end of the external jugular vein, after having tied all branches except that from the gland. Hirudin, in doses of about 0·02 gramme per kilogramme was found to be sufficient to prevent clotting in the cat. The drops of blood were allowed to fall on a slip of mica attached to the end of the lever of a Marey tambour, in connection with another tambour which recorded each drop on the smoked paper. The cervical sympathetic was cut on both sides, so that the gland was supplied with dilator fibres only, conveyed by the

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* 'Arch. f. d. ges. Physiol.,' vol. 12, p. 277, 1876.

† 'Arch. f. d. ges. Physiol.,' vol. 67, p. 469, 1897.

‡ Article "Dépresseur (nerf)," Richet, 'Dictionnaire de Physiologie,' vol. 4, p. 786, 1900.

§ Bayliss, 'Journ. of Physiol.,' vol. 28, p. 276, 1902.

|| Bayliss and Bradford, 'Journ. of Physiol.,' vol. 16, p. 10, 1894.

¶ 'Journ. of Physiol.,' vol. 28, p. 293, 1902.

Chorda tympani. The central end of the vagus, on the opposite side to the gland under observation, was arranged for excitation. As a rule, the animal was eviscerated, in order to diminish the effects of the mere fall of blood-pressure on the rate of flow through the gland. Fig. 1 is a tracing obtained in this way. It will be noted that, in spite of evisceration, there is a large fall of arterial pressure, which lasts for a long time after the excitation ceases. This is usually the case with vaso-motor reflexes in the cat. While the blood-pressure is falling, the drops greatly increase in number per unit time, but later, the effect of the low blood-pressure shows itself by a slowing of the flow. There is, apparently, no lasting vaso-dilatation, as seen in direct excitation of the chorda. The reason for this is, probably, that no saliva is secreted, so that the action of products of cell-activity on the arterioles is absent. In order to save space, the middle part of the tracing has been cut out, amounting to three minutes in time.

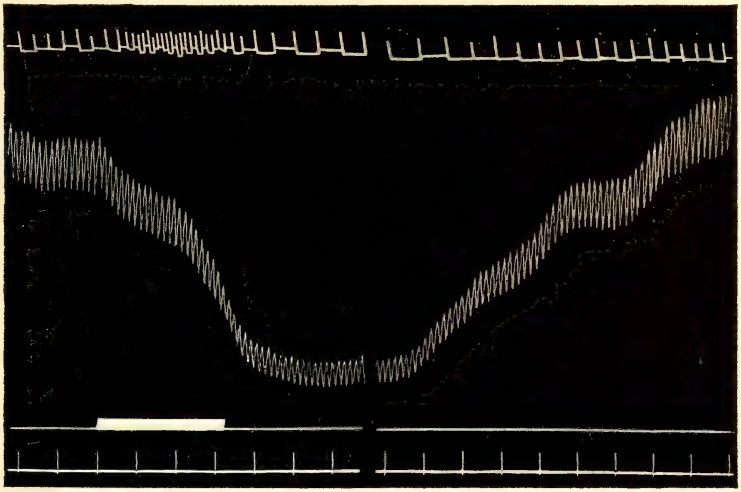

Fig. 1.—Excitation of chorda dilators in depressor reflex. Upper line, drops of blood from gland vein. Middle curve, arterial pressure. Bottom line, time in 10 sec. Zero of blood-pressure, 58 mm. below time line.

A circumstance may be mentioned here that makes the investigation of this, as of other vaso-dilator reflexes, a matter of peculiar difficulty: viz., they are only to be seen while the animal is in the best condition as regards temperature, blood-pressure, etc., *i.e.*, at the beginning of an experiment. It seems improbable that this loss of reflex excitability is due to fatigue, since it has no obvious relation to the number of times the reflex has been excited.

The effect on the submaxillary gland above described is abolished by section of the chorda, but considering the circumstance spoken of in the previous paragraph, too much importance must not be given to this result.

It appears to me that the only objection that can be brought against my interpretation of the experiment is that the increase of blood-flow may be in some way a consequence of the fall of general arterial pressure, the bulbar centres of gland-nerves may be excited by anæmia in a manner like that produced by the injection of extracts of duodenal mucous membrane,* or the rapid fall of pressure may induce a relaxation on the part of the arterioles themselves. Now, in the first place, the time at which the effect appears is not consistent with either of these suggestions. If the fall of pressure were the cause of the dilatation, this would be greatest during, or immediately after, the fall. On putting the question to the test of experiment, by the excitation of the peripheral end of the vagus nerve, I find that there is frequently a short period of increased rate of flow when the blood-pressure returns to normal. It was only seen when there was a rise of blood-pressure following the fall, and was absent after section of the chorda. It appears therefore, to be due to excitation of bulbar centres by anæmia. In order to be able to detect any secretion of saliva, which would only be small, I performed a similar experiment on a dog, but could not observe any secretion. Apparently the secretory centres were not excited by the degree of anæmia produced. It has been found difficult or impossible to excite reflexly electrical changes in glands.†

[*Note added May* 25, 1908.—Professor Langley thinks that objection may be taken to the interpretation of vaso-dilation in the submaxillary gland in response to excitation of the central end of the vagus as representative of a general depressor reflex. The vagus nerve contains afferent fibres from various digestive organs, so that the effect on the salivary gland may well be a special reflex associated with the taking of food and different in its mechanism from genuine depressor reflexes. In order to meet this objection, I have repeated the experiment, but exciting the depressor nerve in the rabbit. In this case also considerable increase of the rate of blood-flow through the gland deprived of constrictors was obtained on excitation. It seems, then, that the vagus effect in the cat may fairly be taken as a true depressor one. Moreover, as shown below, the reflex from the vagus in the cat consists, not only in the excitation of dilators, but also in inhibition of constrictors, so that, in any case, it shows the phenomenon of reciprocal innervation.

Professor Asher informs me that he has obtained excitation of vaso-dilators to the submaxillary gland of the rabbit on exciting the depressor nerve.]

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* Bayliss and Starling, 'Journ. of Physiol.,' vol. 28, p. 348, 1902.

† Bayliss and Bradford, 'Journ. of Physiol.,' vol. 7, p. 224, 1886.

The tongue, especially in the dog, would be expected to be a favourable organ for investigation of vascular reflexes. To my disappointment it was found to give very poor plethysmographic records, so far as concerns changes in its own vessels of an active nature. On excitation of the central end of depressor or vagus there was occasionally to be seen a slight dilatation at the beginning of the fall of pressure, but this was soon overpowered by the passive effect of the lowered arterial pressure. In these experiments the cervical sympathetics were cut on both sides, so that the tongue was supplied only with vaso-dilators.

In the course of experiments made for another purpose two or three years ago, I noticed that in a depressor reflex, the abdominal sympathetics having been cut, there was increase of volume of the penis, enclosed in a plethysmograph. Since the rhythmic contractions shown by the curve were also inhibited, it might be that these contractions, probably due to the retractor penis, were responsible for the changes of volume. I have recently, therefore, repeated the experiment, three times in all.

In the first experiment, although there was a marked effect, it was found, *post mortem*, that only one of the sympathetics had been completely severed, the other was merely crushed. This latter was apparently incapable of conduction, since there was no contraction of the organ on excitation of a sensory nerve nor in asphyxia.

In the second experiment the vaso-dilatation did not appear until the arterial pressure had returned to its original level, so that it might be due to local reaction. This does not seem to have been the case, however, since an equally great and sudden fall produced by cardiac inhibition was not followed by any dilatation.

Fig. 2 was obtained in the third experiment. After a long latent period the blood-pressure begins to fall, and simultaneously there is an increase of volume of the penis. The curve has the appearance of having been cut off at the top; this is, no doubt, due to the piston recorder sticking slightly at this position.

The conclusion to be drawn is that when cut off from the vaso-constrictor centre the penis is still capable of reflex vascular dilatation, which can only be due to excitation of vaso-dilator nerves contained in the pelvic nerves of Langley, the *nervi erigentes* of Eckhard.

It was shown by Winkler,* with some degree of probability, that vaso-dilators to the external ear of the rabbit run in upper cervical nerve-roots. They are, perhaps, posterior root fibres, and, if so, the dilatation is antidromic in nature. The majority of the constrictor fibres run in the cervical sympathetic, although it has been shown by Fletcher* that a few fibres for the tip of the ear run by way of the ramus vertebralis of the stellate ganglion and the third cervical nerve. If the cervical sympathetic is cut, there is, then, a small part of the ear left in connection with vaso-constrictors. I do not think that these fibres play any part in the reflexes obtained under experimental conditions. The ear soon becomes cold and bloodless under anæsthetics and curare, so that only reflex effects of considerable magnitude are shown in the plethysmographic curve. In fig. 3 the result of exciting the central end of the vagus on the opposite side, which, in this case, contained more depressor fibres than the depressor nerve itself, is shown. The cervical sympathetic having been cut, the effect must be due to dilator nerves, apart from the problematic inhibition of Fletcher's fibres, above mentioned. Since the dilators in question are possibly

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* 'Sitzungsberichte d. Wien Akad.,' Math.-Naturw. Klasse, vol. 111, June, 1902.

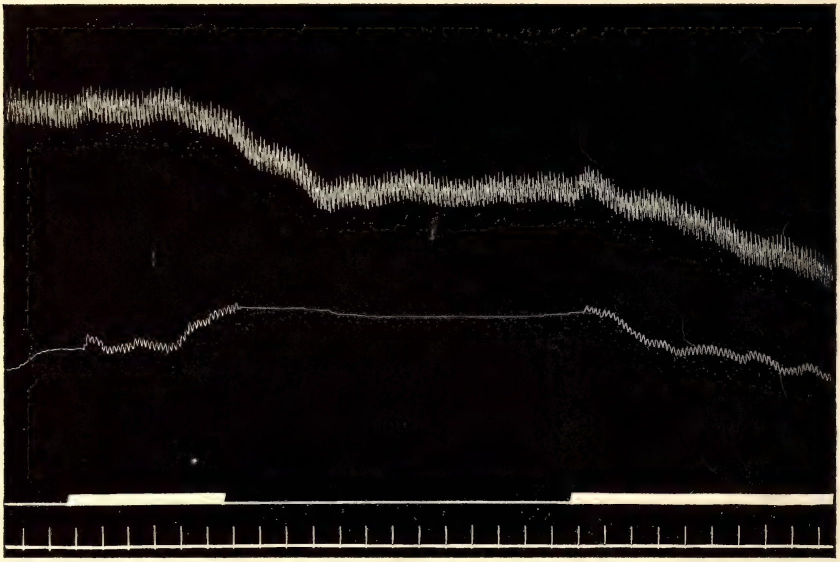

Fig. 2.—Excitation of dilators in pelvic nerves in depressor reflex. Upper curve, arterial pressure. Middle curve, volume of penis. Blood-pressure zero, level of excitation-signal. The second excitation of the vagus central end was without effect on the penis, probably following too closely after the preceding one.†

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* 'Journ. of Physiol.,' vol. 22, p. 259, 1898.

† It will be noticed that in many of the plethysmographic curves given in the following pages small waves appear during the period of excitation. These are merely due to the fact that the exciting coil was placed on the table of the kymograph. The interrupter was in action only during the excitation-period and the slight shaking of the writing lever diminished the friction between it and the smoked paper so that the respiratory waves were traced. This is of use in that it serves to mark out exactly the period of excitation.

antidromic in nature, I did not think it worth while to devote much time to the detailed investigation of the case.

An indirect piece of evidence, tending to confirm the thesis of this section, is derived from consideration of the vascular reflexes in the "eviscerated" animal. In an animal from which all the abdominal viscera have been removed or tied off from the circulation, and in which, also, the abdominal and cervical sympathetics have been cut and the median nerve of one fore-limb prepared for excitation, it is surprising to find that the changes of blood-pressure in vascular reflexes are, in many cases, as large as in the normal animal. When we remember that the only organs retaining their constrictor supply are one fore-limb and part of the other, together with the walls and skin of the chest and upper part of the abdomen, it is difficult to conceive that this small fraction of the total vascular area remaining can be responsible for as large an effect as is obtained in the complete animal; of course, the capacity of the vascular system is very greatly diminished, but the proportion of the part supplied with constrictors to the whole is far less than in the normal animal. On the other hand, the dilator supply of the organs remaining, so far as they possess such, is not interfered with. It is, I think, difficult to avoid the conclusion that the dilators take part in the reflexes in such cases. Since I shall have frequently to refer to the state of affairs in animals in this condition, I shall, for the sake of brevity, speak of these as "dilator" animals. It should be mentioned that no change in the heart-beat could be detected in the reflexes referred to above. To put it shortly, the "dilator" animal possesses the following parts supplied with dilators and devoid of constrictors: head and neck (perhaps excluding brain), hind-limbs, and trunk from middle of abdomen; while the remaining parts have both dilators and constrictors, no part having constrictors only.

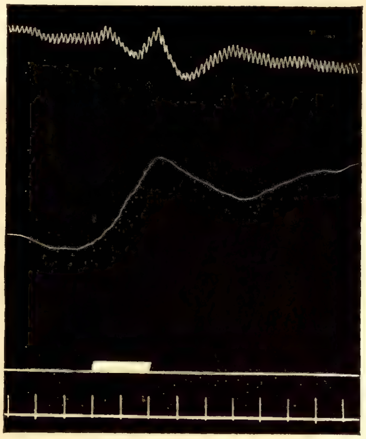

Fig. 3.—Excitation of dilators to ear in depressor reflex. Upper curve, arterial pressure. Middle curve, volume of ear. Time in 10 sec. intervals.

(ii) *Inhibition of Constrictors.*—As already stated, the general opinion is that depressor effects are brought about by inhibition of constrictor tone. But, so far as I am aware, the proof has not been yet given that vaso-dilatation is possible in the complete absence of dilators. Fig. 4 will serve as such a proof. This tracing was obtained from a dog in which, for other purposes, the spinal cord had been transected at the third lumbar segment 27 hours previously. The hind-limbs were, therefore, in connection with vaso-motor centres by vaso-constrictor fibres only. The first excitation in the figure is of the central end of the vagus; a fall of arterial pressure follows, with considerable increase of volume of the limb. Between the two parts of the figure the abdominal sympathetic was cut on the side of the limb under observation. Expansion of the limb is shown, due to cutting off tonic constrictor impulses. The central end of the vagus was again excited, a similar fall of arterial pressure being produced, but this time accompanied by passive *diminution* of volume of the limb. This result is, however, not always to be obtained; it appears that, at all events under experimental conditions, tone of

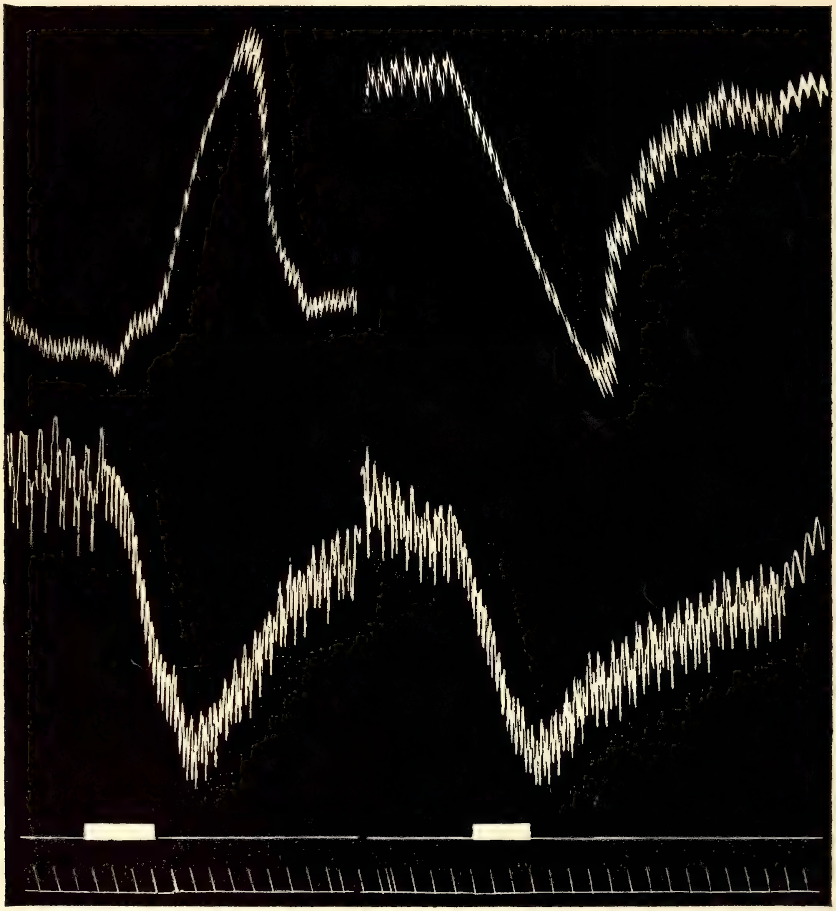

Fig. 4.—Dilatation by inhibition of constrictor tone. Upper curves, volume of hind-limb of dog. Lower curves, arterial pressure. Time in 10 sec. Zero of blood-pressure, 24 mm. below time-signal.

the abdominal sympathetic is sometimes absent, so that after section of the cord no reflex dilatation of the limb can be obtained.*

2. *Pressor Reflexes.*

(i) *Excitation of Constrictors.*—When the arterial pressure rises in response to excitation of the central end of a sensory nerve, it is universally admitted that the effect is due to excitation of vaso-constrictor fibres. But, as in the last case, the possible co-operation of dilators has not been taken into account. I have, therefore, taken a tracing of the change of volume in the hind-leg of a cat, in which the cord has been transected in the middle of the lumbar region, in order to cut off the dilator supply of the part. Fig. 5 reproduces this experiment. At the place indicated by the signal, the central end of the median nerve was excited. The arterial pressure rises, and, at the same time, the limb constricts. The effect is prolonged, as is usual in the cat. Further experiments were deemed unnecessary, as the phenomenon is undisputed.

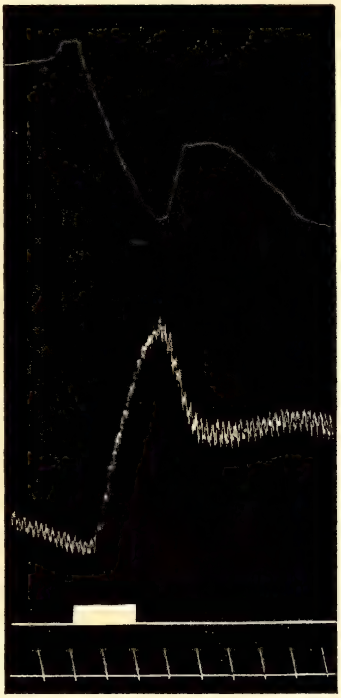

Fig. 5.—Constriction by excitation of vaso-constrictors. Upper curve, volume of leg. Lower curve, arterial pressure. Zero, 25 mm. below time-signal.

(ii) *Inhibition of Dilators.*—This case has proved to be the most difficult one of the four as regards experimental investigation. It is obvious that, in order to be able to show inhibition of tone in the vaso-dilator centre, this tone must first be in existence. Just as in the experiments of Sherrington, where extensor tone was usually produced by decerebration, in my experiments it was necessary to induce tone in the dilator centre by keeping the animal as warm as possible, and, in the eviscerated animal, with renal vessels tied, by the intravenous injection of warm saline, in order to raise the arterial pressure as high as possible. Nevertheless, a considerable number of experiments were of a negative character. At the same time those in which positive results were obtained were precisely such cases as would be expected to possess tone of dilators, viz., those with high temperature and blood-pressure; moreover, the effect was generally obtained, if at all, early in the course of the experiment.

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* *Note added to Proof.*—I have recently observed another case of dilation by inhibition of constrictor tone in the absence of dilator fibres. The blood-flow through the submaxillary gland of the cat is increased in rate by exciting the central end of the vagus on the opposite side, even after section of the Chorda tympani nerve, if the cervical sympathetic is left intact. The effect, as far as it is possible to compare results on different animals, seems to be somewhat less than that obtained when the dilators are intact and the constrictors cut, as described in the previous section.

Fig. 6 is an instance from the ear of the rabbit. The cervical sympathetic being cut, the vaso-motor supply was limited to dilators, with the exception of the few sympathetic fibres to the tip of the ear already referred to.

In fig. 7 an attempt was made to increase the tone of the dilator centre by excitation of the depressor. In order to lessen the effect of the fall of arterial pressure, the peripheral end of the cervical sympathetic was excited along with the depressor; this latter, however, did not seem to be very active, as judged by the change of arterial pressure. It did, apparently, increase the effect of excitation of the median nerve, shown by the difference between the first and second excitations on the tracing. The small effect of the later one may also be due to its following the previous one at too short an interval of time.

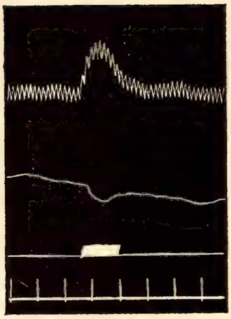

Fig. 6.—Vaso-constriction by inhibition of dilator tone. Median nerve excited. Upper curve, arterial pressure. Zero, 30 mm. below time-signal. Lower curve, volume of ear. Time in 10 sec.

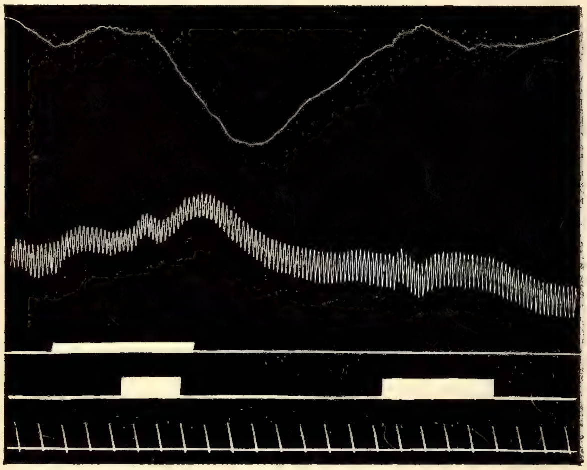

Fig. 7.—Inhibition of dilator tone. Upper curve, volume of hind-leg. Lower curve, arterial pressure. Zero, 40 mm. below time-signal. Uppermost signal, excitation of depressor. Middle signal, excitation of median nerve, twice.

The tongue was disappointing, as before, showing mere passive changes with the blood-pressure; perhaps these changes were rather less than would be expected from the amount of the rise of pressure obtained.

The sub-maxillary gland, with sympathetic cut, drops being recorded as before, showed a distinct slowing of the rate of flow at the beginning of the excitation of the median, but this was rapidly overpowered by the rise of arterial pressure increasing the circulation. It appears that this organ is very sensitive to changes in the general blood-pressure.

On the whole, it seems that, under experimental conditions, inhibition of dilator tone does not play a great part in pressor reflexes. No doubt, under more natural conditions, it has a greater importance. Moreover, from the theoretical point of view, it is of interest to be able to show that it does take place.

Indirect evidence similar to that referred to above, with regard to the magnitude of the vascular reflexes in the "dilator" animal, applies here also. It is rather difficult to find in literature measurements of rise of arterial pressure on exciting central ends of sensory nerves; but, on looking over several curves, I note that 70 mm. Hg is a high value. Bradford* states that the rise obtained from the central end of a dorsal root is of unusual magnitude; on measuring one of the curves he gives, I find that it amounts to 76 mm. Hg. To compare with this, in a "dilator" dog, on exciting the median nerve, there was a rise of 50 mm. Hg, viz., from 130 mm. to 180 mm., and the rise would probably have been greater if the arterial pressure had been lower to begin with, as it appears to have been in Bradford's experiments. It is difficult to avoid the conclusion that inhibition of vaso-dilator tone plays some part in the rise of pressure in the "dilator" animal.

3. *Lovén Reflexes.*

This type of reflex was first described by Lovén in the ear of the rabbit and in the hind-leg of the same animal. It has subsequently been found in various other organs, so that it may fairly be regarded as of general occurrence. Put briefly, when the afferent nerve from any particular organ is excited, there is produced, along with the usual pressor effect on the general blood-pressure, a vaso-dilatation in the organ itself. By this means the maximal supply of blood is sent to an active organ. I have previously shown† that dilator centres are excited, but, as yet, the proof is wanting that constrictor tone is inhibited.

To decide this point, I made the following experiment: In a dog, the dorsal roots of the lumbo-sacral plexus on the left side were divided, and the cord itself transected immediately below the lowest root cut. It was found, *post mortem*, that the roots from the third to the seventh lumbar inclusive had been divided, and the cord was completely severed with the exception of a small piece of the anterior columns. The central end of the sixth root was prepared for excitation. The left hind-leg was placed in a plethysmograph. At first excitation of the central end of the dorsal root produced the usual pressor effect, a reflex *constriction* of the limb, precisely similar to that resulting from the median nerve, so that the Lovén effect appeared to be absent. On carefully raising the root well away from the tissues, however, a different result was obtained. With the same strength of exciting current the rise of blood-pressure was less, a fact indicating escape to the posterior columns of the cord in the previous case, while in the leg, instead of constriction, there was now well-marked *dilatation.* Fig. 8 shows the contrast between the effect of the median and that of a dorsal root carrying sensory impulses from the limb itself. Since the dilators were cut, the effect was due to inhibition of constrictors. We see, then, that reciprocal innervation holds in the case of Lovén, or local, reflexes.

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* 'Journ. of Physiol.,' vol. 10, p. 400, 1889.

† 'Journ. of Physiol.,' vol. 28, p. 292, 1902.

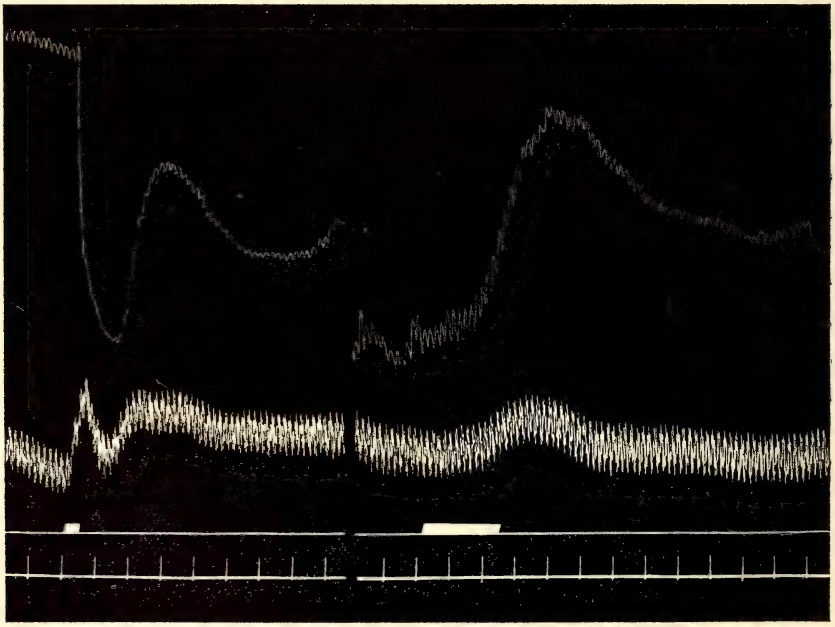

Fig. 8.—Inhibition of constrictors in Lovén reflex. Upper curves, volume of hind-limb. Lower curves, arterial pressure. Zero, 35 mm. below line of excitation-marker. First excitation, median nerve. Second ditto, central end of sixth lumbar dorsal root.

The question was also investigated on the tongue. Excitation of the central end of one of the lingual nerves, the other being cut, while the cervical sympathetics were intact, was followed by constriction of the organ. Either the conditions were not favourable, or the Lovén reflex on the tongue is a matter of dilator excitation alone. The problem of these reflexes is still under investigation.

Piotrowski* has shown that excitation of the central end of the great auricular nerve causes dilatation in the ear, whether the sympathetic be divided or not. Since the latter normally possesses tone, there can be no doubt that in the full reflex there is also inhibition of this tone.†

III. The Action of Strychnine.

It was discovered by Sherrington‡ that the inhibitory component of the "flexion reflex" of the knee is converted by strychnine into an excitation. The phenomenon can only be satisfactorily explained by the hypothesis "that the action of the alkaloid is to convert in the spinal cord in these instances the process of inhibition—whatever that may essentially be—into the process of excitation—whatever that may essentially be."

As the depressor reflex is, at all events in great part, an inhibition of constrictors, Sherrington thought it of interest to test the action of strychnine on this; the result was that, in certain cases, a rise of arterial pressure was produced, instead of the usual fall. Thinking the fact worth further investigation, he suggested to me the continuation of the experiments.

The subject proved to be more complex than it seemed to be at first sight, but ultimately I found that all the various aspects of the action of strychnine could be readily explained on the lines of Sherrington's hypothesis, when the nature of vascular reflexes, as described in the preceding pages, is duly taken into consideration.

The effects of the actual injection of progressive doses of the alkaloid will be discussed in the first place.

It is well known that a considerable rise of arterial pressure is caused by the intravenous injection of a small dose. This is, no doubt, correctly attributed to excitation of the vaso-constrictor centre.* Fig. 9 shows, indeed, that this statement is correct. Vaso-constriction in the intestine is seen coincident with the rise of blood-pressure.

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* 'Centralbl. f. Physiol.,' vol. 6, p. 464, 1892.

† *Note added to Proof.*—It is possible, according to a suggestion made by Professor Langley, that the dilator reflex in the submaxillary gland of the cat in response to excitation of the central end of the vagus, may be of the nature of a local, rather than of a general, reflex. The vagus contains afferent fibres from the alimentary canal and it may be in response to excitation of these fibres that the vessels of the salivary gland are dilated. If this is so, it should, perhaps, be referred to in the present section. In any case, the reflex in question exhibits the phenomenon of reciprocal innervation, as shown in a previous page.

‡ 'Roy. Soc. Proc.,' vol. 76, B, p. 288, 1905.

Supposing that the first dose was not too small, say 5 milligrammes per kilogramme, the injection of a further dose is followed by a *fall* of arterial pressure with a peripheral *vaso-dilatation.* Each subsequent dose is accompanied by the same effect, until complete paralysis of the centres ensues. Fig. 10 is a tracing of the intestinal volume.

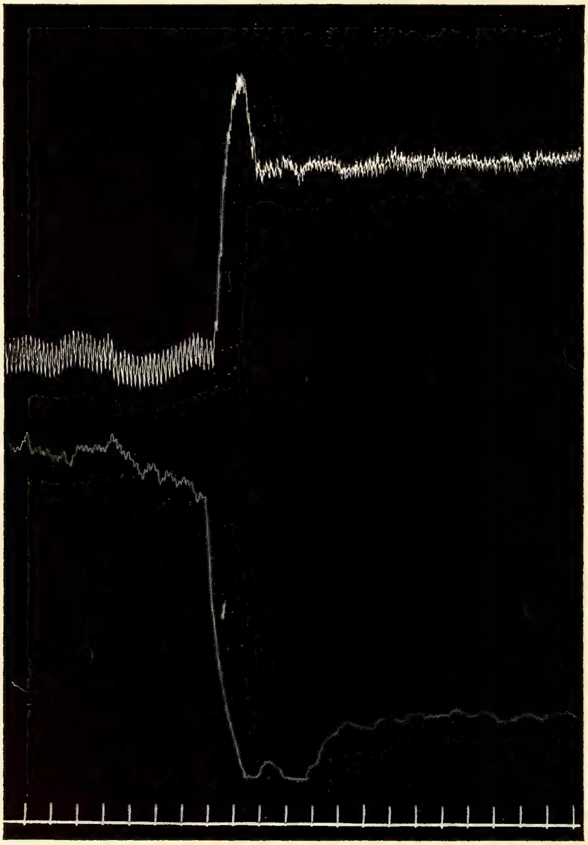

Fig. 9.—Effect of first injection of strychnine. Upper curve, arterial pressure. Lower curve, volume of intestine.

Now what is this fall of pressure due to? There are several reasons for the belief that it is an excitation of dilators. In the first place, the action of strychnine in general is exciting, rather than inhibitory, so that it is quite probable that dilators should share in the general excitation.† Secondly, when the cord is cut in the lumbar region, so that the limbs are deprived of vaso-dilator supply, there is no dilatation in them when a second dose of strychnine is given. But the strongest evidence is the behaviour of the "dilator" animal.

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* *Vide* Cushny, 'Pharmacology,' 4th ed., p. 201, 1906.

† *Vide* Cushny, *loc. cit.*, p. 202.

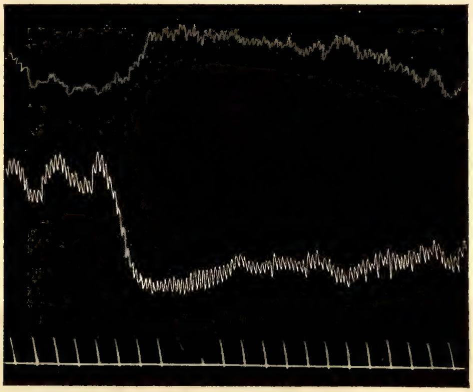

FIG. 10.—Action of a dose of strychnine subsequent to the first. Upper curve, volume of intestine (Cat). Lower curve, arterial pressure. Time in 10 sec.

Here the *first* dose produces *fall* of pressure and *vaso-dilatation* (figs. 11 and 12). In fig. 12 the fall of pressure is preceded by a small rise. In both these cases the organ under observation was deprived of its constrictor supply, so that the increase of volume must be due to excitation of dilators. The drug, in fact, excites both dilators and constrictors indiscriminately, the effect on the

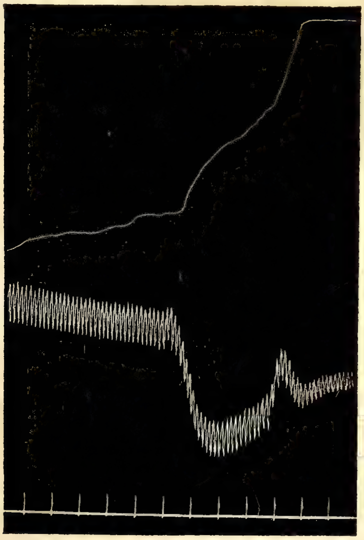

FIG. 11.—Effect of *first* dose in "dilator" animal. Upper curve, volume of ear. Lower curve, arterial pressure.

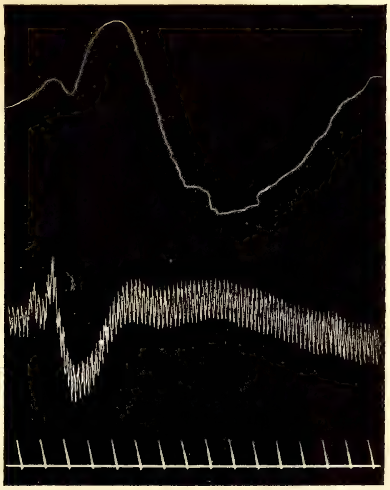

FIG. 12.—As fig. 11, but hind-leg in place of ear.

arterial pressure being due to those in the majority. The reason why the effect, in the complete animal, of the first and second doses is opposite is, in all probability, that the alkaloid, after strongly exciting the constrictor mechanism, paralyses this earlier than it does the dilator. It will be shown later that the toxic action is not on the efferent vaso-constrictor neurone; it may be on the synapse of the pressor fibre or of an intermediate neurone with the cell-body of the former, or even earlier on the afferent side.

In this connection the fact is of interest that, after strychnine, there is no rise of blood-pressure in asphyxia; there is, on the contrary, an excitation of dilators, as shown by fig. 13. The dilatation seen comes on too early to be an effect of raised venous pressure. Since the constrictor neurone itself is not attacked, a fact shown by the persistence of vaso-constrictor effects from the depressor, it follows that the action of asphyxial blood in exciting constrictors is not exerted directly on the efferent neurone itself. In normal animals, I at times observed dilator effects in asphyxia. These do not appear to be due to the direct action of carbon dioxide on the blood-vessels,* since they were not to be seen in organs deprived of their dilator supply.

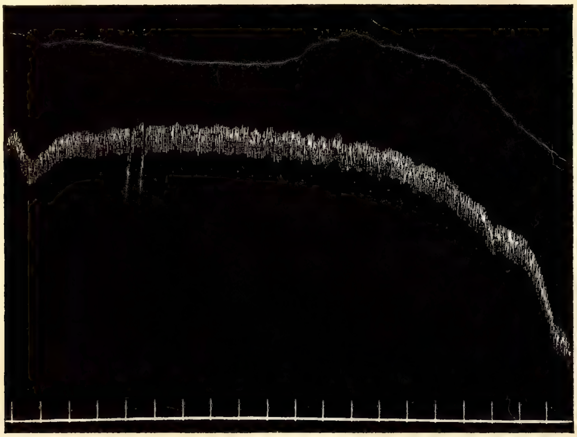

Fig. 13.—Asphyxia after strychnine. Upper curve, volume of penis. Lower curve, arterial pressure. At the time when the tracing begins the artificial respiration was stopped.

That the constrictor mechanism, or rather the afferent excitor part, is paralysed by strychnine in smaller dose than is required by the dilator mechanism, is also confirmed by the fact that the first dose of the drug is usually sufficient to abolish all pressor effects from the central end of an ordinary sensory nerve.

Cocaine produces a rise of arterial pressure by central excitation of constrictors, as usually stated. Accordingly, I find that this drug, injected subsequently to a dose of strychnine, causes a *fall* of pressure, just as a second dose of strychnine itself would have done. On the other hand, bodies acting peripherally, like adrenaline, still produce a rise after strychnine, apparently as great as normally.

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* Bayliss, 'Physiol. Soc. Proc.,' 1901, p. xxxii. In 'Journ. of Physiol.,' vol. 26, 1901.

Vascular Reflexes under Strychnine.

The effect of a small dose is to increase general excitability, not only as regards pressor, but also depressor, reflexes. Fig. 14 is an interesting case of this. In my work on the causes of the depressor fall of arterial pressure, I was never able to obtain a dilatation of the renal vessels of sufficient magnitude to counteract the effect of the fall of blood-pressure so as to produce an actual increase of volume of the organ. In the experiment of which the tracing of fig. 14 forms a part, a similar passive diminution in renal volume was seen on depressor excitation at the beginning. After injection of 0·013 gramme of strychnine, the depressor gave rise to an actual *increase of volume.* As will be seen later, this dilatation is probably due to excitation of dilators, rather than to increased excitability to inhibitory influences.

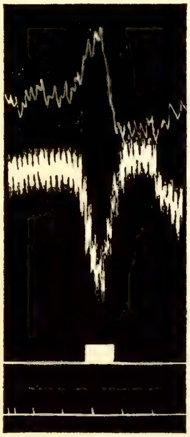

Fig. 14.—Effect of the depressor nerve on the kidney after a small dose of strychnine. Upper curve, volume of kidney. Lower curve, arterial pressure.

As the dose is progressively increased, the depressor nerve being excited between each dose, a change in its effect on the arterial pressure is seen to make its appearance, step by step. The first sign of this change is that the fall of pressure is of short duration only and is followed by a slight rise, which becomes, as the dose is increased, greater in relation to the fall, until it alone is left and finally itself disappears, owing to abolition of all reflex excitability. Fig. 15 gives a series of such curves from the rabbit.

It was necessary in this case to inject 7 centigrammes of the sulphate, in order to produce complete conversion of the fall into a rise. This would be a colossal dose for man, but the rabbit is very insensitive to strychnine. In cats and dogs the effect is brought about by a fifth of this dose. Individual animals, of each species, vary as to the dose required; probably age is the factor concerned.

Although there can be no reasonable doubt that the cause of the rise of

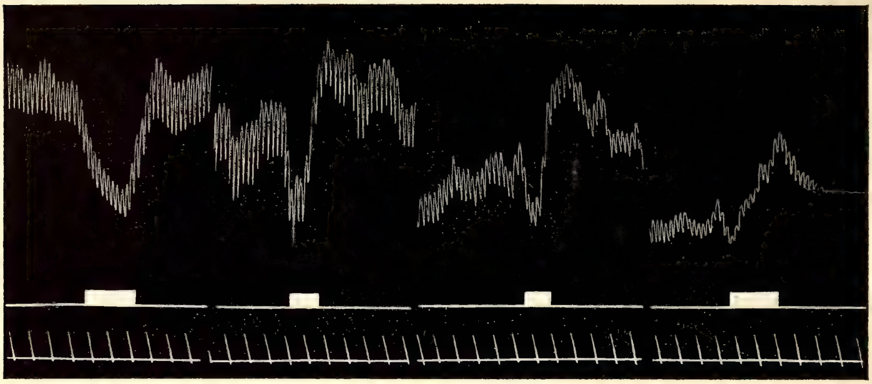

Fig. 15.—Effect of increasing dose of strychnine on the depressor fall in the rabbit.

blood-pressure on excitation of depressor nerves under the action of strychnine is constriction of peripheral arterioles, it is well, at the outset, to make no error as to this fact. Fig. 16 shows that, coincidently with the rise of arterial pressure, there is a constriction of the intestine. Before the injection of the drug, this cat gave the result always obtained on excitation

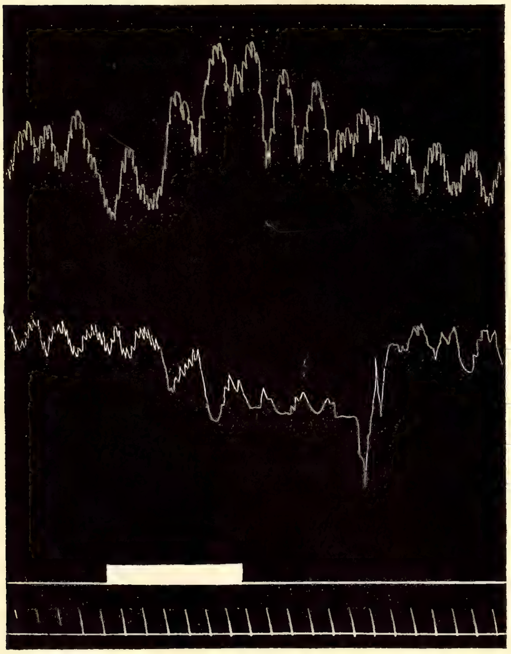

Fig. 16.—Peripheral vaso-constriction produced by depressor excitation after strychnine (Cat). Upper curve, blood-pressure. Lower curve, volume of intestine.

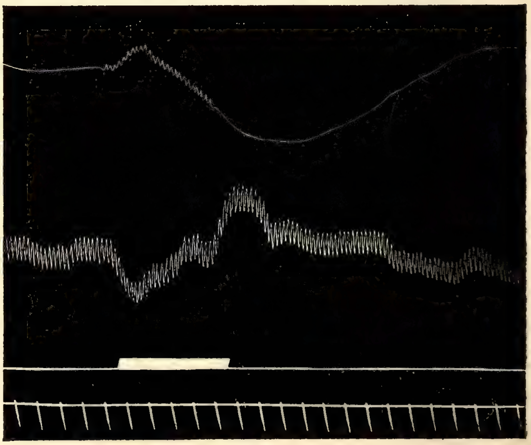

Fig. 17.—Vaso-constriction in spleen produced by depressor excitation after strychnine. Upper curve, volume of spleen. Lower curve, arterial pressure. Zero, 32 mm. below signal of excitation.

of the central end of the vagus, viz., fall of pressure and dilatation in the intestinal vessels. The dose was, as will be seen, not quite sufficient to completely convert the fall into a rise, but the intestinal dilatation is now replaced by constriction. In fig. 17, a similar tracing from the spleen, it is interesting to note that the preliminary short fall of pressure is accompanied by vaso-dilatation.

Although these tracings show that lessening of calibre in arterioles occurs, they do not exclude the possibility of this change being caused by inhibition of dilators, improbable as this may be. For this reason it was necessary to repeat the experiment on an organ in which the dilators can be excluded, such as the hind-leg, which can be deprived of dilators by section of the lumbar cord. Most of these experiments, however, were devoid of result, for a reason which was made clear by subsequent experiments. The animals, in fact, were eviscerated, in order to reduce the effect of the mere change in blood-pressure on the volume of the limb, and at that time I had not sufficiently realised the peculiarity of the vascular reflexes in the "dilator" animal. Finding, as a rule, that it was impossible to obtain the typical reversal of the depressor effect by the usual dose of strychnine, I continued to inject more and more of the drug, only to put an end to all reflexes. The cause of this phenomenon is that dilator excitation, as I have shown, is an integral part of the depressor reflex, indeed, the main part in the "dilator" animal, and this component is not reversed in sign by strychnine, but merely ultimately reduced to zero. In the "dilator" animal, therefore, there will be a fall of pressure as long as any effect is obtained. It sometimes happens, however, by a lucky chance, that the dose of strychnine falls just within the narrow limits between paralysis of the dilator excitation and that of the constrictor effect due to reversal of the normal inhibition. This is a somewhat important fact, since the normal constrictor effect of pressor reflexes is abolished by a small dose of strychnine, as will be seen later. The fact that the constrictor effect due to reversal of normal inhibition is the last to disappear shows that it is a different thing from the ordinary constriction; but our present knowledge is insufficient to explain the difference. In the experiment of fig. 18 the spinal cord was transected in the lumbar region, in order to cut off the vaso-dilators; evisceration was performed and a large dose of strychnine injected. Notwithstanding the bad condition of the animal, excitation of the depressor produced a rise of arterial pressure, accompanied by diminution of volume of the limb, which was later overpowered by the rise of pressure. The result is quite definite, although small.

It did not seem to me worth while to repeat the experiment, without attempting to completely reverse the depressor action. For, supposing that before strychnine the usual effect on the limb was obtained, viz., a dilatation, and that after a certain dose of the drug there was a diminution of volume of the limb but coincident with a fall of arterial pressure, the objection would justly be made that the diminution of the leg-volume was merely the passive effect of the fall of pressure, the proper depressor effect of dilatation being paralysed. Moreover, the absence of a dilator reflex on repetition of the stimulus is by no means uncommon. For this reason I am unable to admit that the mere non-appearance of a dilator reflex is of any value as evidence.

The result of the experiment of fig. 18 is conclusive in showing that the reversal of the normal depressor fall into a rise is a matter effected by the constrictor system and since in normal conditions the depressor inhibits this system, it inevitably follows that, by some means or other, the inhibitory action is changed into an excitatory one.

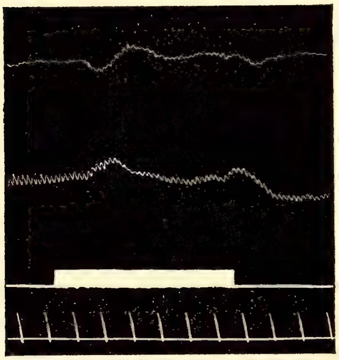

Fig. 18.—Excitation of vaso-constrictors to leg by depressor after strychnine. Upper curve, volume of leg. Lower curve, arterial pressure.

When I demonstrated the rise of pressure from the depressor after strychnine at the International Congress of Physiologists at Heidelberg in 1907, Professor Hans Meyer made the criticism that the result might be due to escape of exciting current to neighbouring tissues, whose reflexes would be more easily evoked under the alkaloid, while the action of the drug on the depressor reflex might be merely that of paralysis. I was able to show that the same result was obtained by pinching the nerve with forceps. It is also easy, by placing electrodes on the surrounding tissues, to show that the suggested explanation is not the correct one; this has no effect at all when the reversal is at its maximum. Indeed, excitation of the trunk of a nerve such as the median at this stage, or even earlier, has no effect on the blood-pressure, a fact which disposes also of another possible explanation of the reversal. This consists in the hypothesis that there are in the depressor trunk a certain number of pressor fibres which come into effect when the depressor fibres are paralysed by the drug.

On proceeding to further investigate the action of strychnine on pressor reflexes, some interesting facts came out. I have already mentioned that very early in the progressive poisoning these reflexes are abolished, so that no effect of any kind is produced in the curarised animal by exciting a pressor nerve. This statement, however, only applies to the complete animal.

Having adopted as a working hypothesis that of Sherrington, I was somewhat surprised, not to say disconcerted, to find that in the "dilator" animal there is, at a particular stage in the action of the drug, a *fall* of blood-pressure on exciting the central end of the median nerve. It is difficult to specify the exact stage, except that a large dose does not seem necessary. Of course, in these experiments care was taken to compare the action of stimuli of the same strength, since it sometimes happens that weak stimuli may cause fall of arterial pressure in the normal animal. This fact is, as yet, unexplained; perhaps it may depend on the presence of depressor fibres which are more excitable. In my experiments this effect was not often met with, perhaps because curare was used. It seems to have some relation to the reflex contractions of voluntary muscle.

Fall of arterial pressure from excitation of a pressor nerve is a characteristic of an advanced stage of the action of chloroform, and is due, in this case, to conversion of excitation of constrictors to inhibition of their centre. Since the general action of strychnine is antagonistic to that of chloroform, it is puzzling to find the same effect produced.

The clue is given by the plethysmograph. Fig. 19 shows that dilatation of the kidney is produced after strychnine under conditions which ordinarily cause constriction and fig. 20 that, under the same conditions, excitation of dilators results. In this experiment, the cervical sympathetic having been cut, the large expansion observed on excitation of the median must be due to

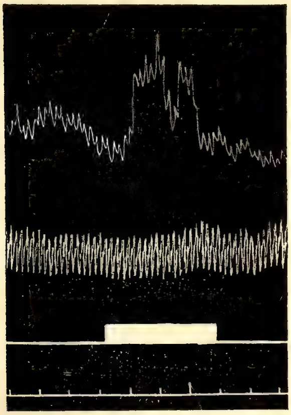

Fig. 19.—Vaso-dilatation in kidney from excitation of the median nerve after strychnine. Upper curve, volume of the organ. Lower curve, arterial pressure.

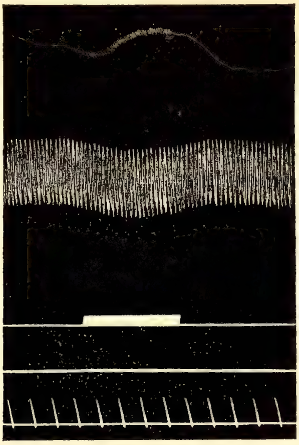

Fig. 20.—Excitation of dilators to ear by "pressor" reflex after strychnine, which has converted the reflex into a depressor one. Upper curve, volume of ear. Lower curve, arterial pressure.

the dilator nerves. Fig. 21 is, perhaps, important, since it shows a similar phenomenon in the hind-limb, after section of both abdominal sympathetics, so that there is no possibility of constrictors playing any part.

These results at once suggest the meaning of the conversion of the "pressor" rise into a fall and of its cause, excitation of vaso-dilators. In the earlier part of this paper I have shown that in the normal pressor reflex there is, along with excitation of constrictors, an inhibition of dilator tone; now, by hypothesis, the action of strychnine is to change in some way a process resulting in inhibition into one of excitation; so that it was really to be expected that the drug would effect such a change that dilator excitation would result from ordinary sensory nerves. Figs. 22 and 23 show the effect of excitation of the median nerve, on the rabbit's ear deprived of constrictors, before and after the injection of strychnine.

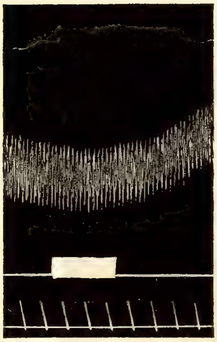

Fig. 21.—Excitation of dilators to hind-leg by reflex from median nerve after strychnine. Upper curve, volume of limb. Lower curve, arterial pressure.

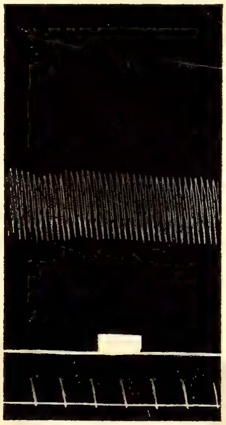

Fig. 22.—Inhibition of dilator tone before strychnine.

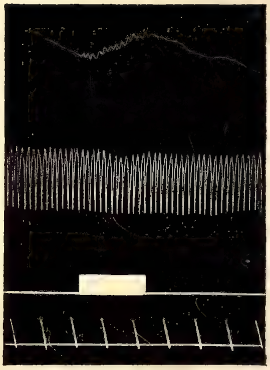

Fig. 23.—Excitation of dilators after injection of the drug. In both figures the upper curves are the volume of the ear, the lower curves are the arterial pressure.

In view of this fact it seems possible that the absence of pressor effects from sensory nerves in the "complete" animal after a comparatively small dose of the alkaloid may not mean total paralysis of the constrictors but a diminution of their excitability only, and that the simultaneous excitation of dilators suffices to antagonise their effect on the general blood-pressure.

The apparent anomaly of conversion of rise of pressure into fall by the action of strychnine turns out to be, in reality, a confirmation of the hypothesis adopted.

With respect to the effect of the drug on the excitation phase of the two kinds of vascular reflex there is little to be said. I have been unable to detect any indication of change of sign before disappearance; which abolition takes place with a less dose of the drug in the case of the constrictor centres than of the dilator centres. It seems also that both the above are paralysed in respect of response to the normal excitation by a less dose than in respect of response to the reversed inhibition, now acting as excitation.

It remains to refer briefly to the action of strychnine on the Lovén reflexes. In the experiment of fig. 8 it was found, after section of all dorsal roots supplying dilators to one limb, that a dilator reflex could still be obtained by exciting the central end of one of these roots. This proves that inhibition of constrictor tone is a factor in these reflexes. In this same experiment, later on, a small dose of strychnine was injected into a vein. On exciting again the same root which had given, previously, dilatation of the leg, a constriction was observed. Accordingly, reversal is produced here as in the general vascular reflexes. The only point to be noted is that the dose of the drug was not sufficient to completely abolish the pressor reflex from the median nerve; it is to be remembered, however, that the dog is very sensitive to strychnine.

Evidence has already been adduced to show that the rise of arterial pressure on excitation of the depressor after strychnine, although caused by excitation of the vaso-constrictor centre, is of a somewhat different nature from that due to excitation of a pressor nerve under normal conditions. In order to obtain more decisive evidence on this point, which seemed of some importance, the following experiment was performed. In a cat the spinal cord was transected at the second lumbar segment, to cut off the hind limbs, one of which was in a plethysmograph, from the dilator centre. Excitation of the median nerve produced the usual result of rise of blood-pressure and constriction of the leg. Five milligrammes of strychnine were now given intravenously. On repeating the excitation of the median nerve no effect whatever was produced on the arterial pressure, although the nerve was dissected out further and the electrodes placed on a fresh spot. If this absence of effect were due to simultaneous excitation of dilators, rather than to paralysis of the pressor synapse with the constrictor centre, the constriction should have shown itself in the limb, where there were no dilators to mask it. Nothing of the kind was seen, however; the limb remained stationary. The conclusion seems justified that the paralysis is a genuine one, although I admit that the result, being of a negative character, is not absolutely cogent. At this stage of strychnine action, it was found that the central end of the vagus still produced fall of pressure, although less than normal, not followed by a rise. Accordingly, a further dose of the drug, 3 milligrammes, was given; this had the effect of completely converting the depressor effect into a considerable rise. At a time, therefore, when the constrictor centre is inaccessible to the normal excitations, it can still respond to the excitatory action of what were previously inhibitory impulses. It appears as if the drug is in some way used up or taken into combination in the inhibitory synapse, in order to perform its function of reversal.

In this connection it is advisable to refer to what may seem a simpler explanation of the strychnine reversal than that given by Sherrington. The usual account of the action of the drug is that it acts upon synapses so as to render those permeable which were previously closed. If we assume that the depressor has connections with the constrictor centre which only became permeable under the action of the alkaloid, and that these are excitatory in nature, the result may be explained. The hypothesis is not in agreement with experimental facts. We have seen that the connections with the constrictor centre are always open in the normal state, but that their mode of action is not excitatory but inhibitory. Moreover, it seems rather that, at all events in this particular case, the action of the drug is of the nature of a block. In the experiment described above, what was especially noticeable was the long latent period of the constriction from the reversed depressor. The rise of pressure from the median commenced, at most, two seconds after the beginning of excitation, whereas that from the depressor under strychnine, in the first excitation, was 22 seconds; in fact I thought that the second dose had been followed by paralysis, so long a time elapsed before any effect was produced. The second excitation had a somewhat shorter latent time, viz., 18 seconds, so that something of the nature of "facilitation" took place. The third excitation had the same latent time as the second. This fact, on the face of it, looks more like obstruction than the breaking down of barriers. It is worth mentioning that this rise of pressure was accompanied by constriction in the limb, so that it was a real excitation of the vaso-constrictor centre.

With respect to the mode of action of the drug, there is one more point worth calling attention to. As shown in fig. 15, this is not done by first abolishing the inhibition and then replacing it by excitation, but by producing, as an intermediate stage, a double effect, so that opposite processes seem to be consecutively induced by one and the same exciting cause. It is possible that this fact may ultimately give a clue to an explanation of the mechanism of the reversal, but at present I am unable to suggest any such.

It has been already pointed out that asphyxial excitation of constrictors is abolished by strychnine in a less dose than that required to abolish the constrictor effect from reversed depressor action. A larger dose is necessary, however, to paralyse the action of asphyxial blood than that which puts an end to the constrictor excitation from the median nerve. The following is the order in which the various effects dealt with in the preceding pages are attacked by the drug:—

1. Constrictor excitation by median or other sensitory nerve.
2. Asphyxial excitation of constrictors.
3. Dilator excitation by depressor.
4. Constrictor excitation by reversed depressor.

My experiments do not enable me to assign a place to the excitation of dilators by reversed median nerve effect.

As to whether this difference of sensitiveness to strychnine implies a different point of attack in each case, I do not feel that we have yet sufficient knowledge of the way the result is produced to warrant an expression of opinion.

IV. THE ACTION OF CHLOROFORM.

In many of the early researches on vascular reflexes it was thought that the action of sensory nerves was of a depressor nature in the rabbit, until Cyon* showed that chloral, used as anæsthetic for these animals, was responsible for the difference in behaviour compared with dogs. This observer, indeed, considered that the action of the drug was due to abolition of function of the cerebral cortex. The view cannot be maintained, since a far larger dose is required to convert the vaso-motor reflex than to paralyse the cortex. I have previously pointed out† that chloroform has the same effect, and is more convenient in practice, since the experiment can be commenced under ether, in order to obtain the pressor reflex, which can then be converted by administration of chloroform; if desired, ether can be returned to and the original form of the reflex obtained.

In experiments with chloroform, the difficulty to be contended with is the paralysing action of the drug on the heart, and, in fact, on all protoplasmic activities. The arterial pressure being low, the centres suffer from anæmia, so that the reflexes obtained are usually small.

Fig. 24 was obtained from a rabbit, in which a plethysmographic tracing was given by the kidney. In the first curve, under ether, excitation of the median produced the typical pressor effect of rise of blood-pressure with constriction of the kidney. Chloroform was now given until the arterial pressure was reduced from 110 mm. Hg to 64 mm.; on repeating the excitation a fall of pressure was seen, accompanied by dilatation of the kidney. In the third curve, ether was resumed and the median excited before complete recovery; there was a diphasic effect in both kidney and blood-pressure—the latter had risen to 88 mm. before the excitation was made.

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* 'Bulletin de l'Acad. des Sciences de St. Petersburg,' December 22, 1870.

† 'Journ. of Physiol.,' vol. 14, p. 316, 1893.

This experiment shows that the fall of blood-pressure obtained from sensory nerves under chloroform is caused by dilatation of arterioles, but does not decide whether this is due to inhibition of constrictors or to excitation of dilators. This point is only to be settled by observing the effect on an organ, such as the leg, in which the dilators have been cut. Since the results are much more easily interpreted when the passive effects are reduced to a minimum by evisceration, it is necessary first to describe the action of chloroform in the "dilator" animal. Now, a similar fate befell my early experiments with this drug to that of the analogous experiments with strychnine; I lost several by pushing the chloroform to total abolition of all vascular reflexes in the vain attempt to obtain a depressor effect from the median nerve. Although it seems difficult to believe, I can see no other alternative but to interpret the rise of arterial pressure seen under maximal

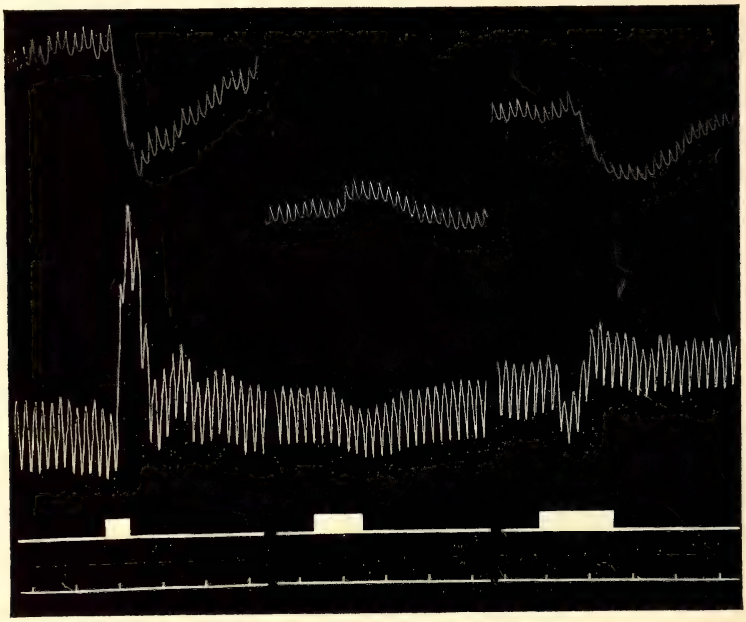

FIG. 24.—Action of chloroform on result of excitation of median on blood-pressure and on kidney. Upper curves, volume of kidney. Lower curves, arterial pressure. Zero is not the same in the three tracings, see text. First tracing, before chloroform. Second, under full influence of the drug. Third, after partial recovery.

doses of chloroform in the "dilator" animal, as due to inhibition of dilator tone.

The first curve of fig. 25 shows the ordinary result of exciting the median nerve in the eviscerated rabbit under ether; rise of blood-pressure and constriction of the limb of which the dilators had been cut. Chloroform was then given in as great an amount as possible without lethal results; the blood-pressure fell by 70 mm. Hg. Excitation of the median still gave a rise of pressure, but now accompanied by *dilatation* of the limb. This means that the process which previously resulted in excitation now results in inhibition. At the same time there are too few constrictors left in the body to counteract by their inhibition the opposite effect on the blood-pressure of the inhibition of dilators present as a normal constituent of the reflex and left intact by the chloroform, which apparently only attacks excitation processes in such a way as to reverse them, while inhibitory processes are unchanged in sign until finally paralysed. The result is that a rise of blood-pressure occurs, produced, not by excitation of constrictors, but by inhibition of dilator tone. The third curve was obtained after return to ether anæsthesia and partial recovery, the arterial pressure having risen by 25 mm. Hg. Excitation of constrictors is commencing to return as a part of the reflex from median nerve.

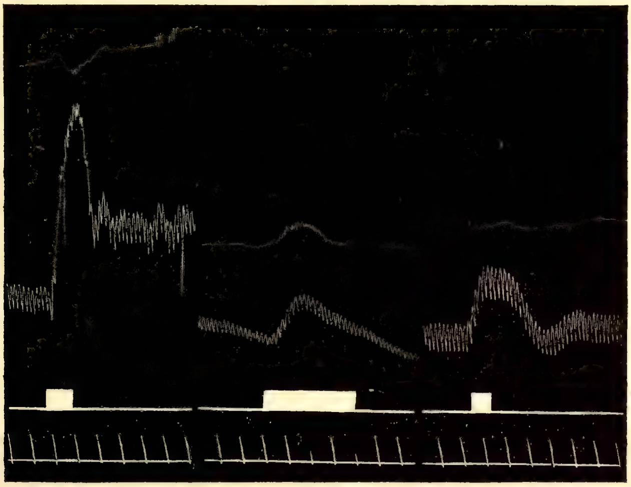

FIG. 25.—Inhibition of constrictor tone by excitation of a pressor nerve under chloroform, etc. Upper curves in each, volume of hind-limb, vaso-dilators cut. Lower curves, arterial pressure. Zero different for each.

The result of this experiment shows the incorrectness of a statement made by me in a former paper to the effect that the depressor reflex from sensory nerves under chloroform differed from the true one from the depressor nerve in that the former was confined to the viscera; in all probability, the experiments on which this statement was based happened to be on subjects devoid of tone in the constrictors to the leg, as often occurs under experimental conditions. This erroneous conclusion serves as a warning against reliance on negative results.

As far as conversion of excitation into inhibition is concerned, we see that the action of chloroform is the opposite to that of strychnine. Since these drugs are regarded as antagonists in general, some experimental results with respect to the action of chloroform on the vaso-motor system will throw some light on the question.

Since in the normal depressor reflex excitation of dilators takes place, it would be expected that chloroform would reverse this effect; so that, in the "dilator" animal, the depressor should cause a rise of pressure by inhibiting dilator tone. Up to the present I have been unable to obtain this result, the normal fall gradually disappears as the chloroform is slowly increased, without giving place to a rise. Whether this is due to the paralytic action of the drug on the centre, or whether the action of reversal is exerted on the constrictor centre alone, I am unable to state. The nearest approach to a positive result was a fall followed by a rise at a certain stage of chloroform action.

The general antagonism of chloral and strychnine is shown in an interesting way in fig. 26. A small rabbit, under the influence of ether, gave curve A on excitation of the anterior crural nerve, the blood-pressure, which was at this time 100 mm. Hg., rose to about 130 mm.; 0·7 gramme of chloral hydrate was then injected intravenously, the arterial pressure fell to 30 mm. and the reflexes were almost abolished (curve B). In curve C is seen the effect of 13 milligrammes of strychnine sulphate. Although this was the first dose, a *fall* of pressure results; that is, the alkaloid acts like a sensory nerve under

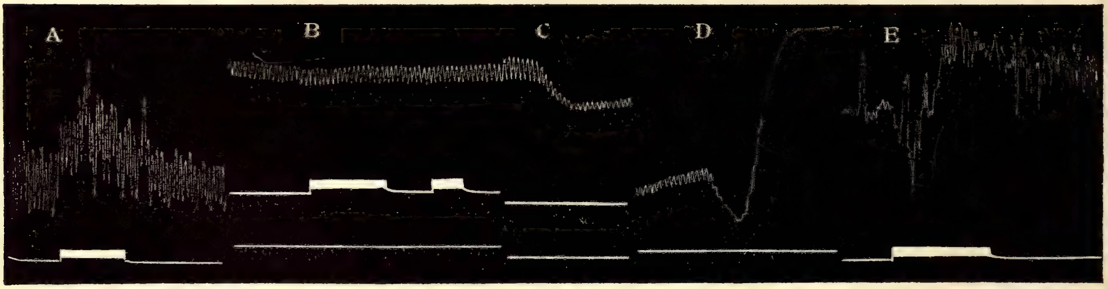

Fig. 26.—Antagonism of chloral and strychnine. A. Effect of excitation of ant. crural on blood-pressure, under ether. B. Same after chloral. C. Effect of first dose of strychnine. D. Effect of second dose. E. Excitation of ant. crural.

chloroform, on the constrictor centre, the normal excitation becoming inhibition. In curve D a second similar dose was given: the first effect was again a fall, the chloral was not yet fully antagonised, but, after a short time, this was effected and the alkaloid now produced its usual excitatory effect. Moreover, excitation of the anterior crural also had its ordinary result, a rise of pressure (curve E).

Asphyxial blood is capable of causing rise of pressure and peripheral vaso-constriction under a dose of chloroform which reverses the effect of exciting a sensory nerve. This fact proves that the action of chloroform is not exerted on the efferent neurone itself, at least not as regards its effect in converting excitation into inhibition.

When electrodes are placed on the floor of the fourth ventricle, over the situation of the so-called vaso-motor centre, it is found that, in the rabbit, chloroform converts the usual pressor effect into a depressor one. It might be thought that this result is at variance with the view here taken as to the action of this drug. It seems unlikely that direct electrical excitation of the efferent neurone should cause inhibition of it. On the other hand, it is much more probable that, by this means, afferent tracts to the centre are excited, rather than the centre itself. So that the experiment is merely a variant of excitation of ordinary sensory nerves.

It is remarkable that the chloroform reversal cannot be obtained in the cat. I have seen a slight rise followed by a fall of pressure, but this latter proved, on examination, to be due to slowing of the heart, although the vagi were cut. It was, presumably, due to the enfeebled heart not being able to work under the raised pressure. In fact, the heart seems to fail before a dose of the drug has been given sufficient to effect change of excitation into inhibition.

As is well known, administration of chloroform is followed by fall of arterial pressure; this is usually attributed to failure of the heart's action, and, no doubt, this is the main factor, but I have several times observed, in plethysmographic tracings of both intestine and limb in the initial stage before the pressure has fallen to any great extent, an unmistakable dilatation of the peripheral vessels. This comes on too early in the fall of pressure to be due to rise of venous pressure and it gives place to a passive diminution of volume as the blood-pressure continues its downward course. It is possible that this dilatation may be produced by direct action of the drug on the arterioles; it is more probable, I think, that it is of central origin and brought about in this way: afferent pressor stimuli are continually being received by the constrictor centre, and are, to some degree at all events, the cause of its normal state of tonic excitation. Under chloroform these stimuli cause inhibition of constrictor tone, and, therefore, dilatation of peripheral vessels.

In view of the special properties of the "dilator" animal, it is not surprising to find that, in this condition, chloroform, if in not too high a percentage in the air respired, sometimes causes a decided rise in arterial pressure, which may continue for several minutes. I can only suggest, in explanation, that in these cases the dilator centre was in tonic excitation before the administration of the drug, and that this excitation was either converted to inhibition or the centre paralysed by the latter, both of which would produce a rise by removing dilator impulses.

On the whole it must be admitted that the effect of chloroform on the vaso-motor centres has not been so clearly made out as that of strychnine. The reason for this is its extremely depressant action on all vital activity, so that it is very difficult to so adjust the dose as to obtain the maximal reversal effect with the minimal paralytic effect.

V. GENERAL REMARKS.

It will, I think, facilitate the understanding of the results described in the previous pages if they are represented in the form of a diagram (fig. 27) on the lines of that of Sherrington.*

When this diagram is compared with that of Sherrington, it will be seen that I have used the name of "reciprocal innervation" in a somewhat extended sense, in that the state of affairs in the vascular reflexes is, in some respects, more complex than that in reflexes to voluntary muscles. The antagonistic impulses, instead of acting on separate muscles, must here be looked upon as affecting one and the same smooth muscle cell. There are also two distinct sets of afferent impulses, pressor and depressor, having opposite relations to the two centres, dilator and constrictor.

There is, indeed, as pointed out by Sherrington himself,† another aspect of reciprocal innervation as concerns the vascular system. The heart and the muscular coat of the blood-vessels may be regarded as antagonistic muscles. The depressor nerve being the afferent nerve from the heart and aorta, will be expected to inhibit the antagonistic muscle, the wall of the arterioles. I hope to investigate the reflexes from this point of view at an early date.

In the present state of knowledge as to what is the essential meaning of excitation and inhibition in the central nervous system, it would be premature to attempt a complete explanation of the phenomena described in the preceding pages. At the same time I think it may be useful to indicate briefly a possible hypothesis which unites what I may call the colloid-adsorption theory of Macdonald and the "drainage" theories of von Uexküll and Macdougall with the synaptic membrane of Sherrington. The doubtful assumption made is the existence of membranes permeable to electrolytes in one direction only. As Overton has pointed out, as a physical structure such a membrane infringes the laws of thermo-dynamics, since it would, by creating potential differences, constitute a perpetual motion machine. I may venture to suggest that its existence may be ensured by the continuous supply of energy from the activity of cell-protoplasm. Macdonald has shown* that the colloids of the axis cylinder, when the nerve is injured, split off electrolytes, which were previously held in a "masked" state, or, as I prefer

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* 'Roy. Soc. Proc.,' vol. 76, B, p. 286, 1905.

† *Loc. cit.*, p. 289.

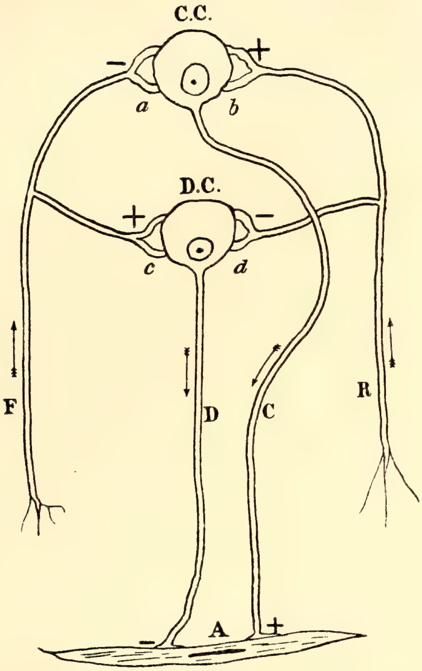

FIG. 27.—Diagram of relations of general vaso-motor reflexes. A. Muscle-cell of arteriole. D. Vaso-dilator nerve-fibre terminating on A, and inhibiting it. C. Vaso-constrictor fibre also ending on A, but exciting it. These fibres arise in the dilator centre (D.C.) and the constrictor centre (C.C.) respectively. F. Depressor afferent fibre, dividing into two branches (or collaterals), one of which (−) inhibits the constrictor centre, while the other (+) excites the dilator centre. R. Pressor fibre of sensory nerve, causing rise of arterial pressure by exciting C.C. and inhibiting D.C. *a*, *b*, *c*, *d*. The respective synapses of these branches with the efferent neurones. The probable intermediate neurones are, for the sake of simplicity, omitted.

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* 'Roy. Soc. Proc.,' vol. 76, B, p. 348, 1905.

to call it, a state of adsorption. We are justified in supposing that the same change occurs in the state of normal excitation. If, under these conditions, a change takes place in the colloids of such a nature that diminution of surface occurs, coagulation for example, adsorbed electrolytes will necessarily be set free. Macdonald also assumes that the opposite process occurs in inhibition. Excitation, then, is associated with increase, inhibition with diminution, of electrolytes. Now in fig. 27 let us imagine that the synaptic membrane at *a* will allow ions to pass from the cell-body to the arborisation, but not in the reverse direction, while that at *c* allows ions to pass from the arborisation to the cell-body only. On arrival of a nerve-impulse, with its setting free of electrolytes, at *c*, these latter can pass freely into the dilator neurone D, causing excitation in it; the constrictor neurone C will not be excited, since the ions are unable to pass into it. Since ions have passed out of *c*, more will travel along the fibre to take their place, and since the membrane at *a* allows their passage from the cell-body of the constrictor neurone C to the fibre F, it is conceivable that such takes place, thus diminishing the concentration of electrolytes and producing inhibition in the constrictor neurone.

On this hypothesis the action of strychnine would be to make the membrane at *a* permeable to electrolytes in the same direction as *c*, so that excitation of both cells occurs.

This is the barest possible outline of a conceivable explanation of the phenomena. No doubt, much modification would be necessary. Moreover, I do not at present see how to account for the chloroform effect on these lines. Expressed shortly, the action of strychnine is to convert the minus signs at *a* and *d* into plus signs: that of chloroform is to convert the plus sign at *b*, and probably that at *c*, into a minus sign. According to the hypothesis sketched above, this statement seems to imply more than a mere summary of the experimental facts, as might appear at first sight.

Fig. 28 is a diagram of the probable relations in the Lovén reflexes.

There is a rather interesting point in connection with these reflexes. When an afferent nerve of a limb, *e.g.*, the median, is excited, vaso-constrictors to the kidney are excited; on the other hand, when the central end of a dorsal root of the kidney area is excited, these same constrictors are inhibited. Is the "final common path" in the two cases the same? Or are there two distinct sets of constrictors? I hope to be able to decide this question in the work on the Lovén reflexes, already in hand.

In respect to the action of strychnine, it will be remembered that it was found by Sherrington that inhibition was not converted into excitation in all cases of reciprocal innervation. Those muscles used to maintain the normal posture of the body were those in the main affected. Now there is an obvious point of similarity between these muscles and the arterial wall, which consists in the circumstance that both are normally in a condition of tone. It is, as Professor Sherrington has suggested to me, a question worth investigation whether there is any special connection between the vaso-motor centres and the labyrinth.

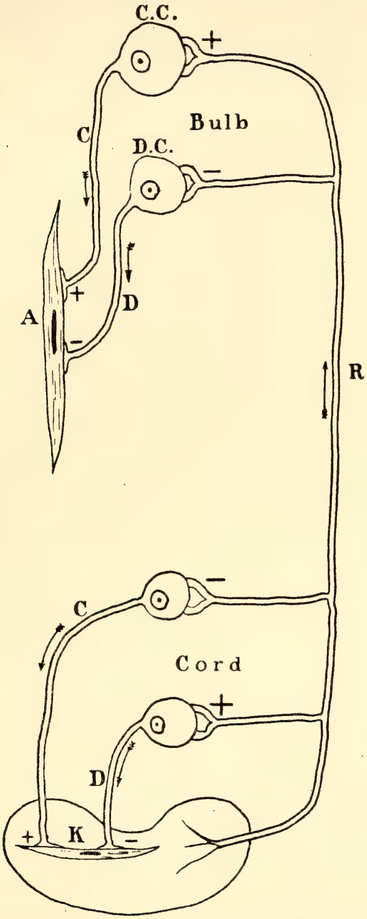


Fig. 28.—C and D. Constrictor and dilator efferent neurones. A. Arterial muscle-cell of body generally. K. Ditto of organ (*e.g.*, kidney) from which the afferent fibre R passes to the bulbar centres, giving off collaterals to the spinal centres. The connections of this fibre are such that it excites constrictor centres in the bulb and dilator centres in the cord, while its relations to the dilator centres in the bulb and the constrictor centres in the cord are inhibitory.

The statement is sometimes made that strychnine causes excitation of constrictors to the viscera and of dilators to the skin. This is not quite the correct way of expressing what happens on injection of the drug.

Constrictors and dilators indiscriminately are excited in all parts of the organism. But, since the skin is relatively more copiously supplied with dilators than the viscera are, it may happen that the net result is vaso-dilatation in the former and constriction in the latter. Moreover, since the volume of blood in the latter greatly exceeds that in the skin and neighbouring structures, the result on the general arterial pressure will be a rise, which itself will further increase the distension of the skin vessels.

During the course of this research I have carefully looked out for any indications of a normal opposition between the visceral and cutaneous circulations. None has been met with. Any reflex involving constriction in the one area was found to have the same effect in the other, and similarly with the dilator reflexes. I refer to the point here since such a state of affairs is still occasionally spoken of as if it had some foundation in fact; it would, obviously, if it existed, require treatment as a case of reciprocal innervation. It is scarcely necessary to repeat that an increase of volume in a limb, for example, in a pressor reflex, must not be taken as a proof of actual relaxation of arterioles; by preventing or diminishing the rise of general blood-pressure, it can be shown that their muscular walls are really in a state of contraction, which can be overcome by a sufficiently great internal pressure.

VI. SUMMARY OF CONCLUSIONS.

1. In depressor reflexes there is, along with inhibition of tone in the vaso-constrictor centres, an excitation of vaso-dilator centres. This has been shown in the cases of the sub-maxillary gland, the penis, the hind-limb, the external ear, and probably the tongue.

2. Correspondingly, in pressor reflexes, along with excitation of constrictors, there is, in appropriate conditions, inhibition of dilator tone. This is, however, more difficult to demonstrate.

3. Similarly, in the local, or Lovén, reflexes, there is also both excitation of dilators and inhibition of constrictors.

4. The action of strychnine is to convert the inhibitory phase of all vascular reflexes into an excitation, so that:—

5. The depressor nerve produces a rise of blood-pressure under full doses of the alkaloid. It does this by exciting the constrictor centre by the same mechanism which normally inhibits it.

6. In the "dilator" animal (see text) under strychnine, pressor reflexes become depressor, in that inhibition of dilators is converted into excitation.

7. Various parts (synapses) of the reflex arc are differently sensitive to the

alkaloid, the synapse of the pressor fibres with the constrictor centre being the first to show paralysis as the dose is increased.

8. In the "dilator" animal strychnine causes a fall of blood-pressure on injection by exciting dilator centres. In the normal animal the first dose causes a rise and subsequent ones a fall of pressure, since the first dose, if not too small, after exciting the vaso-constrictor centre, paralyses the synapses concerned, so that the simultaneous excitation of the dilator centres can now make itself felt.

9. The excitation of constrictors produced by reversal of inhibition is more resistant to the alkaloid than that produced in the normal way.

10. Asphyxial blood does not act directly on the efferent constrictor neurones, since it has no action at a stage of strychnine poisoning at which the depressor still excites constriction, by reversal of inhibition.

11. Chloroform converts pressor into depressor reflexes (in the rabbit), by reversal of excitation of constrictors into inhibition.

12. This effect of chloroform is not exerted on the efferent neurones directly, but at some point considerably earlier in the reflex arc. This is shown by the fact that asphyxial blood causes rise of pressure when excitation of sensory nerves causes fall.

The expenses of this research were partially defrayed from the Government Grant administered by the Royal Society.

[*Note added March* 23, 1908.—Since the preceding paper was written, I have received from Professor Mislavsky, of Kasan, a number of tracings showing a dilatation of the tongue of the dog and cat on excitation of the central end of the vagus, after section of the cervical sympathetics above the superior cervical ganglia. In one case there was no obvious change in the blood-pressure, a fact which perhaps makes the reflex origin of the dilatation somewhat doubtful; in the other cases there was the usual fall. In all cases, as Professor Mislavsky informs me, the effect was abolished by section of the lingual nerves, so that there seems no doubt that it was due to excitation of vaso-dilator fibres. These experiments were performed by Professor Mislavsky in conjunction with his pupil, Mr. Fofanoff.

The results are of interest, in that they bring evidence of dilator-excitation in an organ on which my own experiments had been only partly successful.]

The Action of Resin and Allied Bodies on a Photographic Plate in the Dark.

By William J. Russell, Ph.D., F.R.S.

(Received March 24,—Read May 7, 1908.)

[Plates 10—12.]

In former papers it has been shown that certain metals, woods, juices of plants, etc., have the property of acting on a photographic plate in the dark; that a similar action is exerted by coal resins and allied bodies is proved by the following experiments.

Ordinary resin or colophony is the solid, remaining on the distillation of crude turpentine, and the substance known in commerce as "amber resin" is ordinary resin slightly purified, and is of a lighter colour.

To prove the activity of these bodies it is only necessary to lay them on a photographic plate in the dark, and afterwards to develop the plate in the ordinary way. The plates used in the following experiments were in almost all cases "Imperial Special Rapid." At ordinary temperatures the action is but slow: the contact of resin and plate would have to be for two to three days in order to obtain a fairly good picture. The amber resin is, however, slightly more active than the ordinary resin. If the temperature be raised, and contact be at 30° to 40° C., the action is much more rapid, and three to four hours is long enough to give a good picture. In fact, in four hours, ordinary resin will give as much action at 40° as it would in three days at 15° to 20°. A still higher temperature cannot be used with safety, for then the resin softens and adheres to the photographic film.

Absolute contact between resin and photographic plate is not necessary, for if the plate be held above the resin the action still takes place, and will, in fact, pass through a considerable distance. In one case when powdered resin was placed at the bottom of a glass cylinder and the photo plate on the top at a distance of 120 mm., and in another case when the distance was 210 mm., in both cases after 18 hours' exposure at a temperature of 40°, a dark picture was produced. Another experiment which shows this action of resin was made by filling a glass tube, 1 inch in diameter and 10 inches long, and slightly contracted at one end with small pieces of resin, the tube being held in a horizontal position, and a photo plate placed vertically at 1 mm. from the open end of the tube. On passing a slow current of air through the tube, which was maintained at 40° C., a dark indication of where the air struck the plate was produced in two hours. On continuing to pass air through the tubes the activity of the resin gradually decreases, but if the resin be taken out and again broken up its activity is restored.

If the tube containing the resin instead of being straight is bent at a right angle, and a photo plate be placed below the bend, on passing a slow current of air through the tube a large amount of action is produced upon the plate.

The presence of oxygen appears to be necessary for the action to take place.

Two slabs of resin were placed separately in two desiccators: one was filled with dry air and the other with dry carbon dioxide; a photographic plate was fixed at 1 mm. below the resin plate, and both desiccators were kept at 40° C. for 18 hours. It was then found that, although considerable action had taken place in the desiccator filled with air, none had occurred in the one filled with carbon dioxide.

A very marked and important character of this action of resin is that it is not able to pass through the thinnest sheet of glass or mica or aluminium. Glass 1/200 inch thick and mica 1/750 inch thick absolutely prevents the action passing through. This seems to separate this action from others of a somewhat similar character.

Another important point with regard to the action of resin and other allied bodies is the form of the shadow which they produce. If, for instance, a glass screen is placed in front of a piece of resin on a photo plate the shadow is not bounded by straight lines, but the action, like that of a vapour, creeps in behind the screen, and in time meets from both sides. To prevent this action arising from any side action of the resin plate, a glass tube was filled with resin and directed against the centre of the screen. The experiment was repeated with the same apparatus, and a copper screen and ordinary light. Fig. 1 (Plate 10) shows the effect produced in the two cases.

With regard to other properties of the resin plate, a thin plate acts as energetically as a thick one; thus a plate only 0·017 inch thick gave a picture of the same density as one 0·29 inch thick.

To obtain a suitable slab of resin for experiment it is best to melt the resin and cast it on a bright metal plate, and afterwards, to free the surface which has been in contact with the metal from air-bubbles, to pass a gas flame over it.

Another way of using resin for experiments is to dissolve it in alcohol and saturate a card or paper with the solution and allow it to dry. Even very dilute solutions may be used: a card which has been soaked in an alcoholic solution containing 0·25 per cent. of resin will give a dark picture, and with solutions of only 0·125 and even 0·086 per cent. of resin, faint pictures may be obtained. Again, another way of using resin is to pour the alcoholic solution on to a glass plate and allow it to dry there. There are, of course, other solvents which may be used in place of alcohol.

A card prepared with an alcoholic solution of resin was placed in the dark slide of a camera, and the light of an arc lamp focussed upon it for five minutes. The card was then put up with a photo plate at 55° for one hour. A good and dark picture of the arc was obtained.

If resin be heated to a temperature of 40° to 50° for a short time it does not affect its activity, but if the heating be continued for 20 or 30 hours it slightly diminishes it. At higher temperatures the action is more marked; for instance, at 140° the activity of the resin is much decreased after only four hours' heating, and, although resin may be fused without appreciably diminishing its activity, still, if it be kept in a liquid state for three or four hours, its activity is much decreased. An interesting experiment is easily made with a slab of resin owing to its brittleness. A weight placed on the slab cracks it in all directions; this can be slightly warmed on the under side so as to prevent its falling to pieces, and then on putting it up with a photo plate for a short time a dark picture of the cracks is obtained (fig. 2, Plate 11). Another interesting experiment shows that the activity existing in resin can be transferred to a non-active body, making it as active as the original resin. A glass vessel was nearly filled with crushed resin, and a piece of inactive Bristol board placed on the top of it, at a distance of 5 mm. above the resin. This was left for a week at ordinary temperature, then on putting the Bristol board in contact with a photo plate at 55° C. for five hours a dark picture was obtained.

There are other ways in which this action of resin may be diminished or destroyed; for instance, by the action of sulphur dioxide. A slab of resin was broken into two pieces; one was placed for five minutes in a saturated solution of sulphur dioxide, then very thoroughly washed and dried; the other piece was treated in the same way, but with water alone, and both pieces were put up at 40° for 18 hours. The one which had been washed with water alone gave a dark picture, and the one treated with the sulphur dioxide gave no picture.

It has been shown in a former paper that wood, after exposure to sunlight, has its power of acting on a photographic plate in the dark much increased, and that this increase of activity is not permanent, but gradually passes away. Resin acts in the same way: expose it to sunlight or to the arc light, and then bring it in contact or proximity to a photo plate, and it will be found that its activity has been greatly increased. Fig. 3 (Plate 11) shows the picture given by the resin in its ordinary state, and after exposure to the arc light for half an hour. In one experiment a slab of resin was exposed to a bright July sun for 5 seconds, and this caused no increase of activity; but exposed for 15 seconds and a slight increase occurred, and after an exposure of 30 seconds the activity of the slab had greatly increased; but on a still longer exposure no further increase took place, so that in about 30 seconds the resin was charged to its maximum amount. Another experiment, when the exposures were for 1 minute, 5 minutes, and 15 minutes; the 1 minute and the 15 minutes gave similar results. If the arc light be used in place of sunlight the same kind of action occurs, only more slowly. In one experiment the exposures were for 5 seconds, 15 seconds, and 30 seconds, and no marked increase of activity took place, but after 60 seconds' exposure a great increase was evident. With another sample of resin exposed to an arc light it was found that it required 5 minutes' exposure to obtain its greatest amount of activity.

If amber resin, in place of ordinary resin, be used, it requires a longer exposure to light to charge it to its greatest amount. Resin is, however, a body which varies so much in composition and constitution that exact measurements cannot be relied on for different specimens, and the above experiments are only intended to show the nature of the action which occurs. The effect of heat on resin in its ordinary state has already been described; if, now, a resin slab, charged to its maximum by exposure to light, be heated to 55° for only one and a-half hours, all this extra activity is destroyed and it returns to its original state of activity.

This increased activity induced by light acts generally in the same way as the original activity of the resin: it is destroyed by sulphur dioxide and does not pass through glass, mica, etc. In all cases of stimulating resin by the action of light a short interval occurs after the application of the light and before the increase of activity begins; when once begun, the increase takes place rapidly and it soon becomes charged to its maximum, so that longer exposure produces no further increase of its activity.

In order to ascertain which rays of the spectrum were most active in producing this change, a spectrum obtained from an arc lamp, with a quartz prism and lens, was allowed to fall upon a slab of resin for one and a-half hours, and this gave, after contact with a photo plate for two hours at 40° C., evidence of action having taken place where the blue rays had fallen on the resin and not elsewhere. On placing slabs of resin in double bell jars with different coloured liquids, the blue, a solution of ammonia sulphate of copper, and the red, potassium bichromate, it was found that, even after an exposure of one hour in strong diffused light, the resin which had been exposed to the blue light had been strongly acted on, and gave a good dark picture, while the one exposed to the red light gave no increase of activity. The amber resin acted in the same way. A beam of blue light or of red light thrown on a slab of resin or a card saturated with resin gave the same results. On exposing resin under different coloured glasses the same effects were produced: after an exposure of one hour to bright daylight, under a blue glass, the resin became very active, under a red glass no change took place, and under a green glass there was only a very slight increase of activity.

It thus appears that the action of light in this respect, on resin, is similar to its action on wood, as described in a former paper. This increased activity of the resin slowly passes off, even at ordinary temperatures, on keeping in the dark or in red light. A slab was exposed to the arc light for one hour and then cut up into eight pieces. One piece was put up with a photo plate at once at 40° for two hours: it gave a good dark picture; the other pieces were kept in the dark, at ordinary temperatures: after three days a piece was tested, the picture it gave was only slightly lighter than the former one. After nine days, again, a loss of activity had occurred and the same was the case after 18 days. The experiment was carried on for nine months, and at the end of this time although the picture it gave was much fainter than the first one still it was slightly darker than the picture it would have given before exposure. If the resin be only slightly stimulated by exposure to bright daylight, the same gradual decrease of activity was traced. In red light the decrease was apparently the same as in darkness.

Although glass and some other bodies are opaque to the action of resin, porous bodies, of course, allow the action to pass through; for instance, with ordinary paper, if a slab of resin be placed behind it, a very good sharp picture is obtained; if, however, the paper be highly glazed and dressed, it is perfectly opaque. With ordinary papers interesting pictures, showing their structure and water-mark, and stencil pictures are easily obtained.

If paper be treated with different substances in solution, it is made more or less transparent. As a general rule it would seem that acid salts, such as the sulphates, which do not act on the photographic film, make a paper opaque, but that neutral salts do not alter its transparency. If a paper be dried by warming it, it becomes rather less transparent.

The principal constituent of resin is said to be an acid, known as abietic acid. It is not a body which has been very thoroughly examined, but it has the property of acting on a photographic plate in the dark to a remarkable extent. It can be obtained by dissolving resin in alcohol and passing hydrochloric acid gas into the solution; the acid then separates out in a crystalline form. By repeating this process it may be purified, and will then have a melting-point of 156° C. It is with an acid so prepared that the following experiments have been made. If a small glass vessel be nearly filled with the crystalline acid and a photo plate be laid on the top, not touching the acid, at ordinary temperatures, after two hours no action will have occurred, but after 18 hours the plate will give a strong dark picture. If, however, the acid be kept at a temperature of 40°, then a fairly good picture can be obtained in two hours, and with longer exposure a very dark one.

Thus it acts in the same way as resin, and has about the same amount of activity. Exposed to sunlight or to the arc light, its activity is much increased. Exposed to the arc light for an hour, it gives a good and dark picture, and even on an exposure of half that time a picture only slightly lighter is obtained; in fact, in little more than half an hour it is charged to its maximum amount. Light acts upon it as it does on resin.

The acid dissolves readily in alcohol, and if the solution be allowed to evaporate on a glass plate, it gives a film suitable for experimenting with. Paper saturated with the solution becomes very active. The acid also dissolves in ether, benzene, chloroform, etc., and behaves in the same way as with an alcoholic solution.

If the acid be heated to 100° it slowly loses its activity; after eight hours' heating the picture it gives is only slightly fainter than before heating, but after 56 hours' heating it has become much fainter, and after being heated for 152 hours it has lost entirely its power of acting on a photo plate.

If the acid be fused it becomes quite inactive, but its activity is restored if it be powdered, or if its surface be rubbed with sand-paper—in fact, if the smooth surface be broken up. If exposed to sunlight or to the arc light its activity is much increased, and different coloured rays affect it as they do resin. Exposed under blue or white glass to six hours' sunshine it gives a dark picture, but under a red glass only a faint one. All the metallic salts of this acid are entirely without action on a photo plate: neutralise a solution of the acid with potash or soda and its activity has gone, and there is the same loss of activity with the copper and the lead salts, whether in solution or in the solid state. Decompose the metallic salts and the liberated acid is as active as before.

To purify the acid the lead salt, which is very insoluble even in alcohol and other organic liquids, was boiled several times with pure alcohol, and afterwards treated with sulphuretted hydrogen, the acid well washed and dried, and recrystallised from alcohol, and it was found to be quite as active as before this treatment. Another specimen of the acid was treated with an insufficient amount of alcohol to dissolve the whole of it. After boiling and digesting for a considerable length of time the undissolved acid was filtered off, washed, and dried, and was found to be quite as active as before this treatment, so that neither process of purification affected the activity of the acid. If the fused and inactive acid be simply exposed to light it will again become active. If the activity of turpentine depends to any appreciable extent on the presence of abietic acid, then if it be treated with an alkaline body its activity should be decreased. Turpentine is known to be a very active body, and a plate placed about one-eighth of an inch above it will, even at ordinary temperatures, in three hours give a black picture. Some turpentine was allowed to stand for 18 hours with a small amount of solid caustic potash; this was then filtered off, and the liquid distilled and put up with a photo plate for three hours; no trace of action was visible. Another photo plate was placed above the same turpentine solution and allowed to remain for 18 hours; even then only a very faint action took place. Another specimen of turpentine was shaken up with magnesium oxide and allowed to stand for 24 hours. The clear liquid gave a much fainter picture after this treatment. The same occurred when dry sodium carbonate was used, but lead acetate had no action on the turpentine.

Amber, although classed as a resin, differs so much from the substance already described that it was of much interest to ascertain how it would act under similar conditions. It is a remarkable substance, known from the earliest times, and has been used for many purposes.

Quarried at one time, like a stone, it was naturally looked upon as a mineral, but is now known to be of vegetable origin: the exudation of certain trees, probably mostly coniferous ones, which have been buried in the ground for ages. Even in the Green-sand formation some amber has been found. At the present time the principal supply of amber comes from the shores of the Baltic, but a small amount is still picked up on the east coast of this country.

If a piece or pebble of amber, either in its rough state or cut so as to give it a flat surface, be laid on a photographic plate in the dark, no action takes place, even if the contact be continued for 18 hours and the temperature be at 40° to 50° C., thus differing from resin. This has been tried with a large number of specimens from different parts of the world, and with true amber has always been found to be the case.

There are many bodies closely resembling amber in appearance, chiefly resins, which act strongly on the photo plate, and although readily distinguished by an expert in the subject, can easily be mistaken for true amber. It often happens that a piece of amber, after long exposure to a plate, will develop on it small spots of action; these local actions are produced by fine cracks in the amber, which frequently occur, and it is above the opening of these cracks that the action takes place. If the amber be laid for a minute on a hot surface the opening of the cracks fills up and the action ceases. This resembles the action of resin, and apparently points to the collection of volatile matter within the cracks.

Another way of showing that, although a flat surface of amber does not act on a photo plate, still there is a trace of active vapour connected with it, for if powdered amber is placed in a glass dish with a plate above it, but not necessarily touching the powder, after the usual exposure a dark picture is produced. Amber, as is well known, is practically insoluble in alcohol, but in all cases a very small amount of some substance dissolves out of amber; now if this substance be collected by filtering the alcoholic solution and evaporating it to dryness, the residue is found always to be a very active body and gives a dark picture, thus a lingering indication of the amber's origin seems to be indicated.

Following the same line of experiments as that applied to resin, amber was exposed to sunlight and to the arc light, and its activity was found to be much increased. Four pieces of amber were exposed to sunlight for different lengths of time, namely, for two, three, five, and seven hours. After two hours only a very faint picture was produced; after three hours the picture was much darker and strongly outlined; after five hours it was still darker, and after seven hours a very dark picture was produced. The arc light acts in the same way. A specimen of good amber was cut into four pieces, and all of them were exposed at the same time, at a distance of 9 inches from the arc light: one piece for one hour, another for two hours, and the other two for respectively four and six hours. All of them were afterwards put up with photo plates at 55° C. for 18 hours. The amber exposed for one and for two hours did not act on the plate; the one exposed for four hours gave a considerable amount of action, and the one exposed for six hours gave a dark picture. Another experiment of the same kind showed that the amber became slightly active in two hours, and was much increased after four hours, but after six hours and even after ten hours but very slight increase of activity occurred. As amber is a body which varies so much in constitution and composition, the action of light on it will vary slightly with every sample. For instance, five pieces of amber, all from different sources, were exposed at the same time for three hours to an arc light: two of them gave dark pictures, two only faint pictures, and one no picture at all.

Amber, like resin, if stimulated to increased activity by the action of light, gradually loses this increased activity on keeping it in the dark or in dull light, but for a long time retains a slight amount of its increased activity. If, however, the amber be heated, this loss of activity takes place rapidly, even when heated to only 50° C., and if a flat surface of it be brought in contact with a piece of heated metal for one minute the amber loses entirely its activity. It has already been shown that resin is stimulated especially by the blue rays of the spectrum; the same thing occurs with amber. Specimens of different ambers were exposed both to sunlight and to arc light under different coloured glasses, and it was always found that under the blue glass it became strongly active and that under the red glass it remained quite inactive, and if black glass and colourless glass were used the black glass acted like the red glass and the white one like the blue, only rather stronger. When double bell jars with coloured liquids were used in place of coloured glasses, exactly similar results were obtained. One experiment of this kind was continued for four months and gave the same result.

Lignite, jet, and peat have also been tested in the same way as resin and amber. Two specimens of lignite from the Museum of Practical Geology, Jermyn Street: one an ordinary brown coloured piece, the other a sample from Tasmania; both were quite inactive and light did not stimulate them to action; even the alcoholic extract was inactive. Another specimen from Nigeria was also inactive, but one from Bovey Tracey was slightly active, and a specimen of "Brown coal" from Victoria, after an exposure of 44 hours, was found to be also very slightly active. Several specimens of jet from different sources were tried. None of them, if simply laid on a photographic plate and warmed, gave any action, but if powdered and a plate placed at 1 mm. above it, at 55° for 18 hours, gave a faint picture. Again, if powdered jet was extracted with pure alcohol, the small amount of dissolved matter evaporated to dryness gave a dark picture. So that jet, although not in ordinary conditions an active body, still in the form of powder has the property of acting on a photo plate. Light does not appear to have the power of making it active.

Graphite from Ceylon did not act on a photo plate. A specimen of peat was found to have the property of acting on a photo plate, but its activity was not increased by exposure to light.

One other substance belonging to this class of bodies, namely coal, remained to be examined, and it was interesting to find that all ordinary coals, if brought into contact with a photo plate at a temperature of about 50°, were capable of acting upon it and giving a clear and distinct picture; so sharp are these pictures that they may be enlarged five or six times and still show clearly all the details. Fig. 4 (Plate 10) is the picture of a vertical section of a Nottingham coal enlarged three times. The vertical section of a coal gives usually a more interesting picture than the horizontal section. Figs. 5 and 6 (Plate 11) show vertical and horizontal sections of a Seaham coal. Through the kindness of Dr. Teall and Mr. Strahan, of the Museum of Practical Geology, I have had the opportunity of examining coals from different localities. Taking first the specimens of English coals, they all seem to be active, that is, have the power of acting on the photographic film in the dark. The best way of trying them is, first to saw off a piece from the rough block, and then rub it down first on coarse sand-paper and then on fine, till the surface is flat and true, then on laying this flat surface on a photo plate at about 50° C. for in most cases about 18 hours, but in some cases it may be well to continue the contact for as long as 48 hours, a good picture is obtained. If the coal contains much water, it must be dried, either by heating it for a short time at a temperature of about 40° C. or by drying it over sulphuric acid.

In place of using a slab of coal, it is sometimes convenient to use it in the form of powder, and this is done, as in previous cases, either by simply placing the powder on the photo plate or by filling a small glass vessel with it and placing the photo plate on the top, either in contact with the powder or at a small distance above it. As long as the coal is used in form of a slab and is fairly dry, its action is very uniform, different pieces of the same coal giving pictures of the same density; but when the coal is in powder, a small amount of moisture modifies the density of the picture to a very considerable extent.

The effect of slightly heating a coal is shown by the following experiment:—Four samples of a Seaham coal in powder were treated as follows: One sample was at once put up with a plate, and gave a fairly good picture; another was heated for 24 hours at 100° C., and gave a much darker picture; a third one was heated at 150° for the same length of time, and its picture was much lighter, only slightly darker than the first one; and the fourth sample was heated for 24 hours at 200°, and gave no picture.

In another case the heating was continued for only three hours at 200° and it gave a faint picture. If the drying be effected by placing the powder over sulphuric acid, phosphorus pentoxide, or solid caustic potash, it seems in many cases to increase the activity of the coal to a very considerable extent, so much so that some coals which under ordinary conditions give only a faint picture can be made to give a dark one. But on the other hand there are coals which are not altered by this process of drying. One specimen of coal, a Seaham coal, powdered, was exposed 11 times in a glass vessel over sulphuric acid, each time for 24 hours, without any diminution of its activity;

but if the coal was exposed to the air for 24 hours its activity considerably decreased, but was restored by again placing it over sulphuric acid.

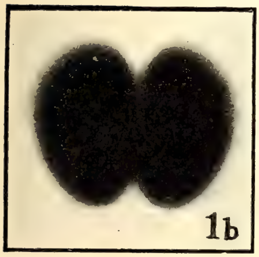
Coals exposed to sunlight or arc light do not perceptibly increase in activity, as many other bodies do, nor does the small amount of substance dissolved out of them by boiling alcohol appear to be active.

The following pictures are fair samples of coals from different English beds: figs. 7 and 8 (Plate 12) are both from South Wales. Fig. 9 is a Nottingham coal, fig. 10 a Derbyshire one, and fig. 11 is from Lancashire. In all cases the deposit of vegetable matter in long or short strips or patches is clearly shown and well defined, and the presence of vegetable matter appears diffused through the mass of the coal.

Although there must necessarily be a strong resemblance between coal pictures, still it may prove that a certain specific and recognisable character belongs to coals from different beds. For instance, judging from the few specimens which have been examined, the South Wales coals appear to have their active strata fine and near together, whereas the coal from Derby and Nottingham has active strata which are much thicker and very sharply defined; but considering the small number of experiments made, this may be purely accidental. The pictures, however, clearly show differences in coals; for instance, all the anthracites that have been examined have given pictures different from the foregoing: they are fainter in appearance, the structure they represent is more complicated and the active matter more evenly distributed through the mass of the coal, as shown in fig. 12. There always appear to be cracks in anthracites and these cracks are always white. There is also another curious point with anthracites: if they are dried over sulphuric acid the picture they give is much darker than the picture obtained in the ordinary way, fig. 13. Only a few Cannel coals have been examined: these gave pictures in character like the anthracites but with less detail and not so dark. Fig. 14 is a picture of the well-known Boghead Cannel coal.

From coal plants of different kinds and from different localities no pictures have been obtained. If the soft powder so common in bituminous coals and known as "Mother of Coal" is carefully removed and tested it is always found to be very active. The large amount of diffused action on the top of fig. 4 is owing to this substance; also the fibrous substance so often present and easily removed from coal is also very active, but the hard glistening surface of coal is only slightly active. Of the coals which have been examined, a Boora coal from the Lower Oolite, a Jurassic coal from Mexico, and some Argentine coals and a Tertiary coal from India are ones which have been found to have little or no action on the photo plate. No

1a



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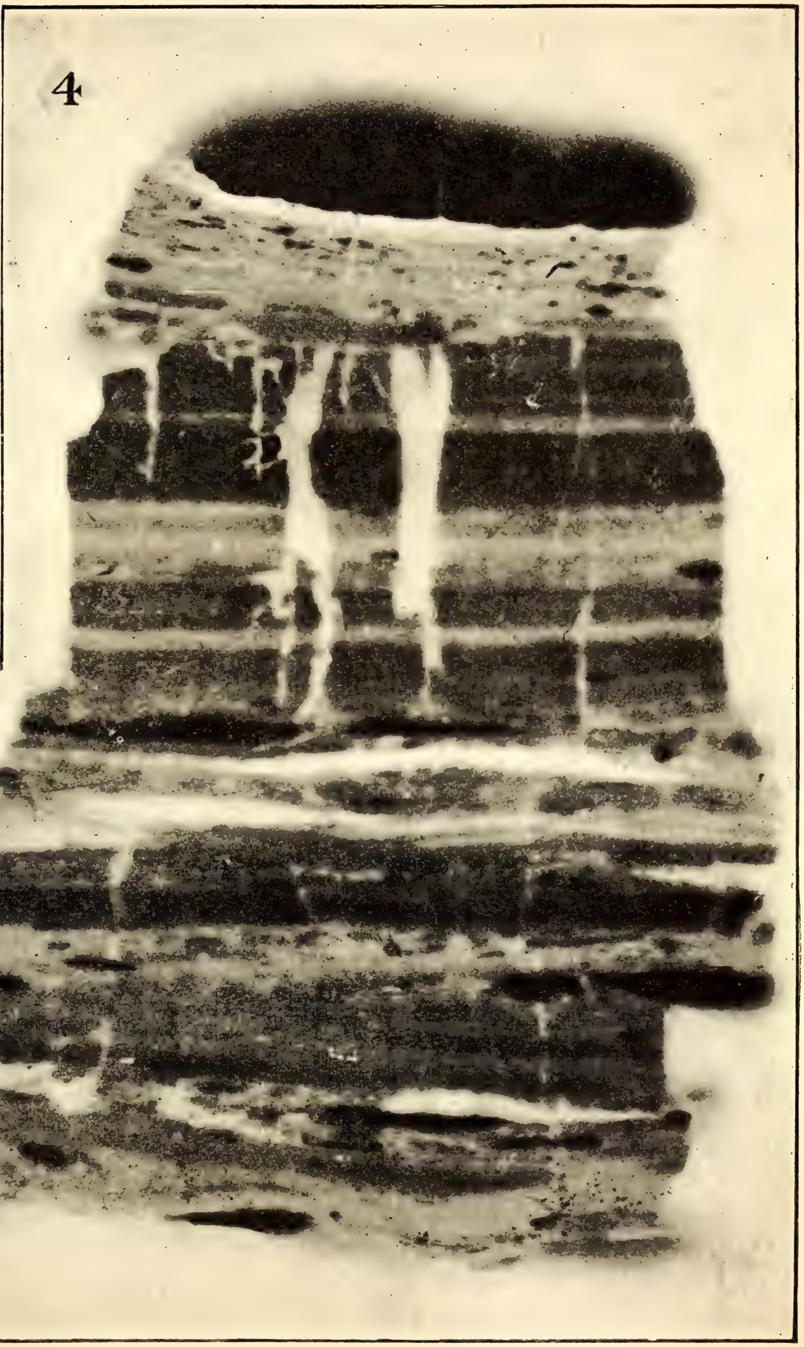

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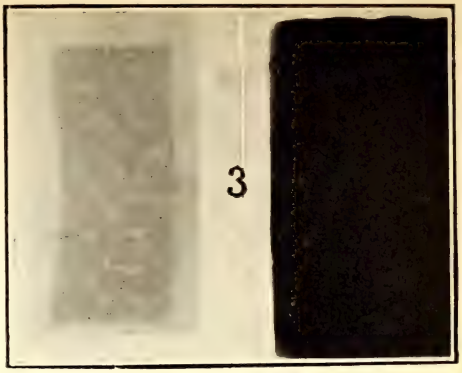

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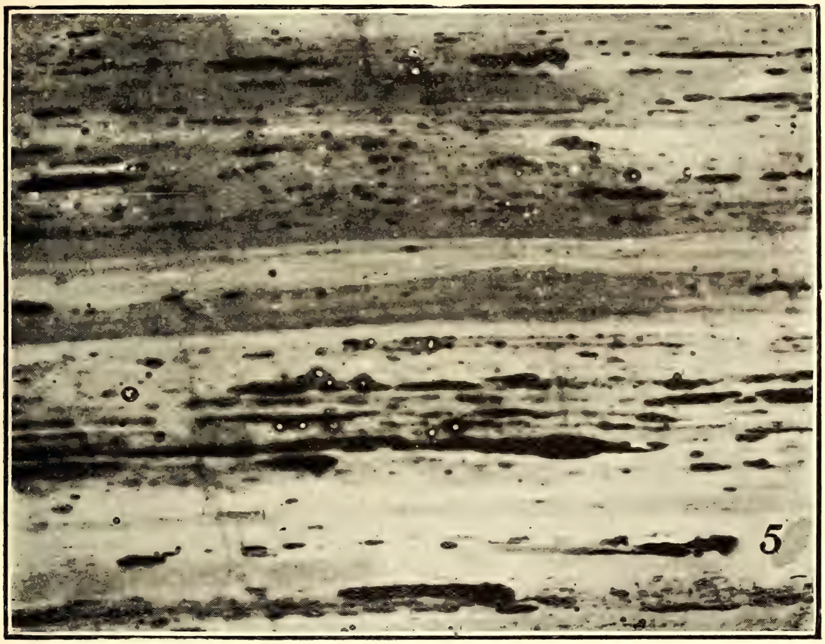

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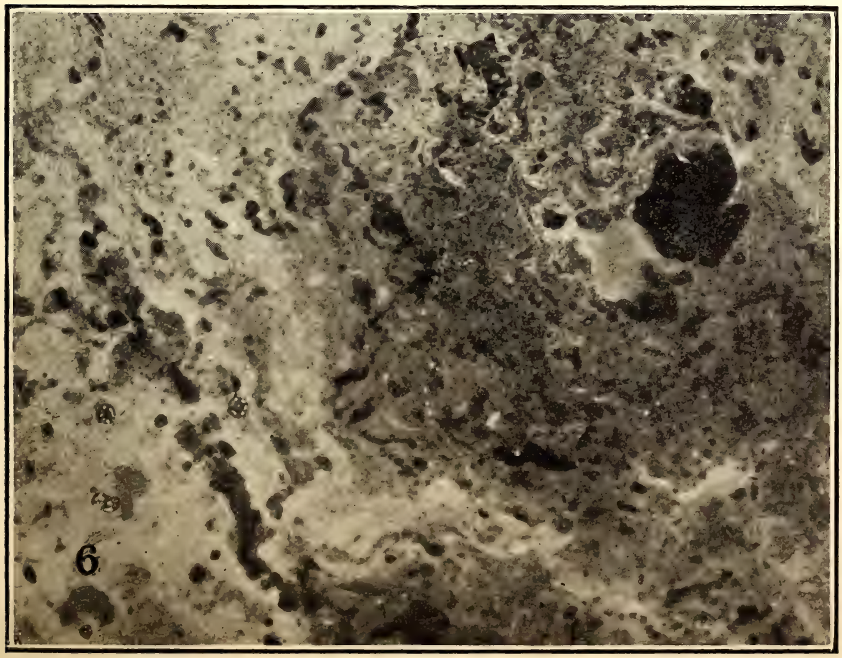

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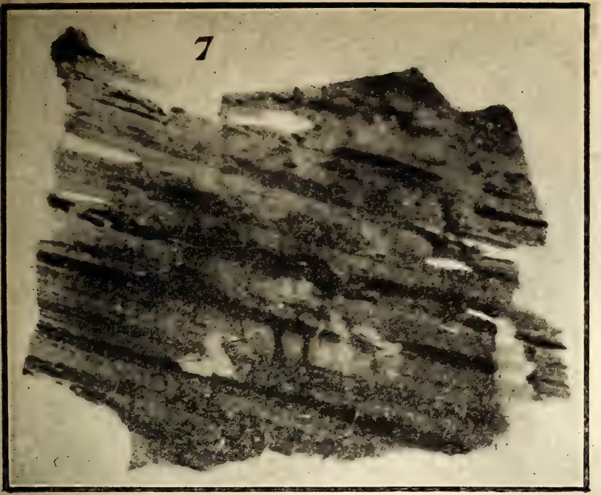
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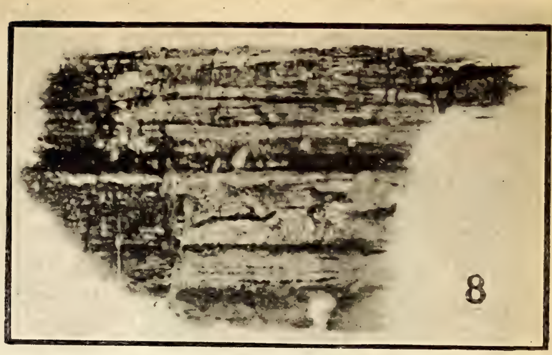
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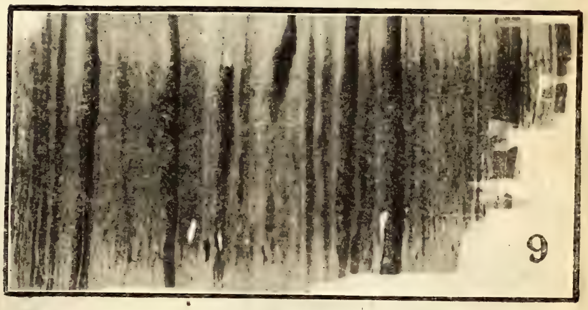
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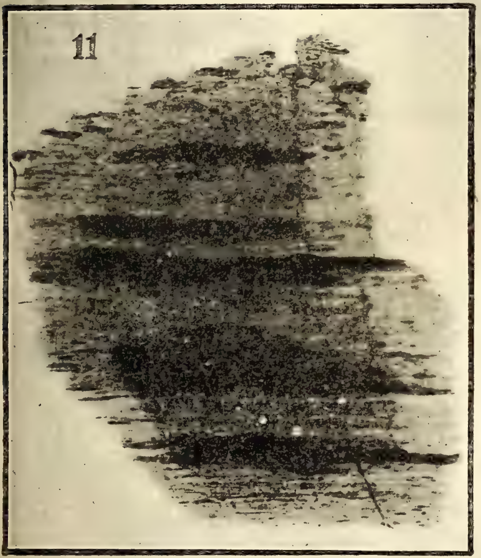
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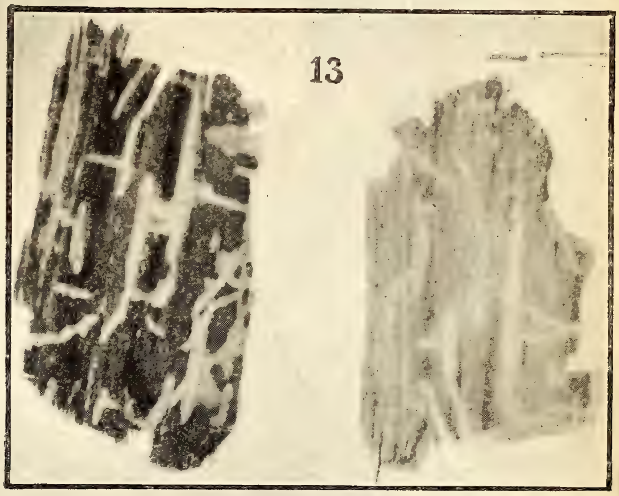
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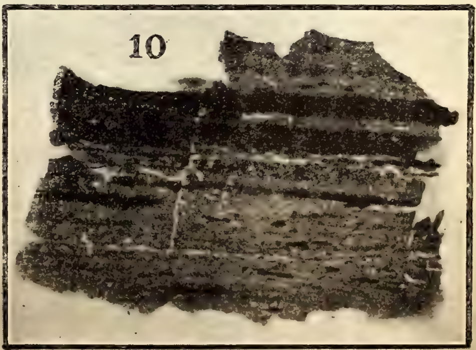
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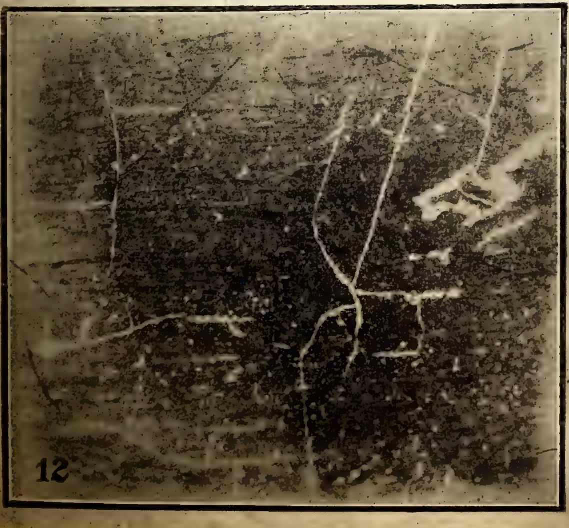
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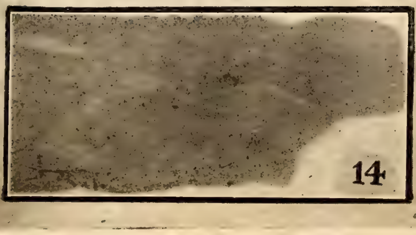
14

doubt the long exposure of small specimens in a museum may affect their activity.

The foregoing experiments indicate the nature and to some extent the results which may be obtained by allowing coal to draw its own picture on a photographic plate, and in the hands of a geologist may help to explain the process of its formation.

With regard to the nature of this action on photographic plates in the dark, it has been suggested in former papers that it is owing to the presence of hydrogen peroxide, and that the effects described can be imitated by means of this body. It now seems that actions of this same kind are obtainable from many other bodies, but still bodies of the same kind, and these additional experiments strongly indicate that the action is produced by a vapour rather than by any form of radio-activity. For instance, it is shown that the shadows thrown by resin are not bounded by straight lines, but curve round a screen; that the action is not capable of passing through glass, mica, or aluminium foil, even of extreme thinness, and does not affect an electrical field. The action can pass along a glass tube, even when it is bent at a right angle, and may be swept out of a tube by a slow current of gas; and, further, an experiment described above shows that the activity of resin can be transferred to a piece of perfectly inactive Bristol board, which will then give a black picture. Further, no action takes place in an atmosphere of carbon dioxide. On the other hand, resin dissolved in an inactive liquid, such as alcohol or petroleum spirit, causes it to become active.

The action which strong light has in increasing the activity of many bodies is important. For instance, it has been shown that pith may be in contact with a photographic plate at 55° for 48 hours and no trace of action is visible, but if the pith be exposed to sunlight for two or three hours it will then give a dark picture. The same action occurs with old printing, with pure india-rubber, etc., and many bodies which under ordinary conditions are but slightly active become very active after exposure to bright light or simply to blue rays.

My thanks are due to my assistant, Mr. Bloch, who has made all the photographs and given me much aid in carrying out the experiments.

The work has been carried on in the Davy-Faraday Laboratory of the Royal Institution.

On Some Features in the Hereditary Transmission of the Albino Character and the Black Piebald Coat in Rats.—Paper II.

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(Communicated by Professor A. D. Waller, F.R.S. Received January 14,—Read May 21, 1908.)

This paper contains a further record of experiments which were begun at the same time as those recorded in my first paper.* The method of interpreting results in the present paper is the same as that used in the first one, and is based upon the same working hypothesis, *i.e.*, Bateson and Punnett's "Presence and Absence" hypothesis and Cuénot's theory of colour. The cost of these experiments has been defrayed by a grant from the Government Grant Committee of the Royal Society.

The Experimental Matings.

(1) (a) *Cr* 4 × *Cr* 4 = *Albino* × *Albino*.

Twenty pairs of albinoes were mated. There was a total offspring of 174 individuals, all albinoes. Some of these albinoes had a near albino ancestry, while others had a pigmented one. The details of the ancestry may be seen upon reference to the Table of Ancestry, pp. 390—391.

(b) "*Ghost*" *or Zygotic Patterns in Albinoes.*

I have already, in the first paper, given an account of the "ghost" pattern as shown in the offspring of one mating (experiment 74). I have now to describe a second case. In experiment 19A there was a total offspring of 15, in two litters of six and nine individuals. The first litter was at a much earlier date than the second, and I did not pay any particular attention to the appearance of the coat in the individuals of this litter. By the time the second litter was born, I had discovered the existence of the "ghost" pattern in albinoes. It is possible, therefore, that the first litter showed it, but since it is only a transitory matter of a few weeks, it will be unobserved unless looked for at the particular period at which it appears. Of the nine albinoes born in the second litter, two died quite young, and the remaining seven all showed the "ghost" of the self-pattern. One of these "self-ghost" individuals was mated with a Cr 5 (see experiment 37C)* and gave a coloured offspring, all of the self-pattern. The young consisted of nineteen "Irish" *b* individuals, obtained in three litters. Since the self-pattern is dominant to the piebald, it is clear that the pigmented piebald parent (Cr 5) could not have been carrying the self-pattern determiner. The albino parent is therefore the only source through which it could have come. And it should be noticed that one of its G-Ps. (experiment 74), and three of its G-G-Ps. (experiments 71 and 66A) had the self-pattern coat.

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* "On Some Features in the Hereditary Transmission of the Self-black and the "Irish" Coat Character in Rats.—Part I," 'Roy. Soc. Proc.,' B, vol. 80, 1908.

Now, on the basis of gametic purity and segregation, it is expected that one-half of the 15 offspring in experiment 19A of which the albino parent described above was one will carry the self-pattern determiner. By an ocular demonstration, corroborated by a subsequent breeding test, the expectation is thus shown to be fulfilled.

(2) *Cr* 4 × *Cr* 5 = *Albino* × *Piebald Black-White.*

(a) *Heterozygous Piebald* × *Albino.*

Experiments 29, 30, 31, 33, 34, 36, 37 *and* 37A.

The expectation in this cross is 1 Cr 4 : 1 Cr 5. The result is 44 Cr 4 + 55 Cr 5. The high proportion of the Cr 5 type is due to mating No. 30 (p. 391). If this is eliminated, the figures are 32 Cr 4 + 33 Cr 5. The disturbance of the proportions is due to the last two litters of this mating. They are:—

1st litter = 3 Cr 4 + 4 Cr 5
2nd " = 4 " + 4 "
3rd " = 2 " + 8 "
4th " = 3 " + 6 "

In experiment 34, one of the four albino offspring showed the piebald "ghost" pattern in its coat.

(b) *Homozygous Piebald* × *Cr* 4.

Experiment 32.

The expectation is that all the offspring shall be Cr 5. The result is 14 Cr 5.

(c) *Piebald* × *Cr* 4 *of more Complex Constitution.*

Experiments 35, 37B, *and* 37C.

In these crosses the result will depend upon the nature of the albino parent. If it be of cgBSsP constitution and the Cr 5 parent carries c recessive,

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* See also Pedigree Chart 1, in first paper.

Table of Ancestry and Offspring.

The symbols and abbreviations have the same signification as the corresponding ones in the first paper, *loc. cit.*

Expt.	G-G-G-P.	G-G-P.	G-parents.	Parents.	Offspring.							
					Cr 1.	Cr 2.	Cr 3.	Cr 4.	Cr 5.	Cr 6. "*b.*"	Cr 6. "*a.*"	Cr 7.
	See Expt.—	See Expt.—	See Expt.—									
1	—	—	—	Cr 4 ♂ 1	—	—	—	13	—	—	—	—
	—	—	—	Cr 4 ♀ 2								
2	—	—	—	Cr 4 ♂ ?	—	—	—	3	—	—	—	—
	—	—	—	Cr 4 ♀ 151								
3	—	—	—	Cr 4 ♂ 150	—	—	—	7	—	—	—	—
	—	—	—	Cr 4 ♀ 151								
4	—	—	—	Cr 4 ♂ 300	—	—	—	12	—	—	—	—
	—	—	—	Cr 4 ♀ 301								
5	—	—	—	Cr 4 ♂ 400	—	—	—	24	—	—	—	—
	—	—	—	Cr 4 ♀ 401								
6	—	—	—	Cr 4 ♂ 100	—	—	—	4	—	—	—	—
	—	—	—	Cr 4 ♀ 101								
7	—	—	3	Cr 4 ♂ 153	—	—	—	11	—	—	—	—
	—	—	3	Cr 4 ♀ 154								
8	—	—	25	Cr 4 ♂ 256	—	—	—	8	—	—	—	—
	—	—	25	Cr 4 ♀ 257								
9	—	—	1	Cr 4 ♂ 8	—	—	—	15	—	—	—	—
	—	—	25	Cr 4 ♀ 258								
10	—	—	1	Cr 4 ♂ 9	—	—	—	8	—	—	—	—
	—	—	1	Cr 4 ♀ 10								
11	—	—	1	Cr 4 ♂ 11	—	—	—	7	—	—	—	—
	—	—	1	Cr 4 ♀ 12								
12	—	—	1	Cr 4 ♂ 13	—	—	—	15	—	—	—	—
	—	—	1	Cr 4 ♀ 14								
13	—	—	1	Cr 4 ♂ 15	—	—	—	5	—	—	—	—
	—	—	1	Cr 4 ♀ 2								
14	—	—	3	Cr 4 ♂ 155	—	—	—	3	—	—	—	—
	—	—	3	Cr 4 ♀ 156								
15	—	—	5	Cr 4 ♂ 405	—	—	—	7	—	—	—	—
	—	—	5	Cr 4 ♀ 404								
16	—	—	5	Cr 4 ♂ 408	—	—	—	6	—	—	—	—
	—	—	5	Cr 4 ♀ 407								
17	—	—	25	Cr 4 ♂ 263	—	—	—	3	—	—	—	—
	—	—	25	Cr 4 ♀ 264								
18	—	—	25	Cr 4 ♂ 272	—	—	—	3	—	—	—	—
	—	—	25	Cr 4 ♀ 273								
19	—	47 and 5	61	Cr 4 ♂ (1)(4)750/150/403	—	—	—	5	—	—	—	—
	—	47 and 5	61	Cr 4 ♀ (2)(4)750/150/403								
19A	5, 23, and 48	71 and 66A	74	Cr 4 ♂ (2)(5)52/53/(3)(2)450/6/408	—	—	—	15[1]	—	—	—	—
	5, 23, and 48	71 and 66A	74	Cr 4 ♀ (3)(5)52/53/(3)(2)450/6/408								

25	—	—	—	Cr 5 ♂ 250	—	—	—	7	17	—	—	—
	—	—	—	Cr 5 ♀ 251								
26	—	—	25	Cr 5 ♂[2] 252	—	—	—	—	8	—	—	—
	—	—	25	Cr 5 ♀ 253								
27	—	—	25	Cr 5 ♂ 260	—	—	—	3	4	—	—	—
	—	—	25	Cr 5 ♀ 259								
28	—	47 and 25	59	Cr 5 ♂ (5)(9)750/152/252	—	—	—	—	3	—	—	—
	48, 25, and 3	62 and 20	38	Cr 5 ♀[3] (1)(1)1000/263/(2)55/(4)153/154								
28A	23 and 5	71	34	Cr 5 ♂ (2)(4)(2)450/6/408/(5)(2)450/6/408	—	—	—	6	13	—	—	—
	23 and 5	71	34	Cr 5 ♀ (1)(4)(2)450/6/408/(5)(2)450/6/408								
29	—	—	25	Cr 5 ♂ 252	—	—	—	8	10	—	—	—
	—	—	25	Cr 4 ♀ 256								
30	—	—	—	Cr 5 ♂ 250	—	—	—	12	22	—	—	—
	—	3	14	Cr 4 ♀ (3)155/156								
31	—	—	26	Cr 5 ♂ (4)252/253	—	—	—	5	3	—	—	—
	—	—	1	Cr 4 ♀ 11								
32	—	—	25	Cr 5 ♀ 255	—	—	—	—	14	—	—	—
	—	—	3	Cr 4 ♂ 156								
33	—	—	25	Cr 5 ♂ 261	—	—	—	5	5	—	—	—
	—	3	7	Cr 4 ♀ (1)153/154								
34	—	23 and 5	71	Cr 5 ♂ (4)(2)450/6/408	—	—	—	4[4]	4	—	—	—
	—	23	71	Cr 4 ♀ (5)(2)450/6/408								
35	—	—	25	Cr 5 ♂ 262	—	—	—	8	3	2	—	—
	—	—	1	Cr 4 ♀ 17								
36	—	—	25	Cr 5 ♂ 268	—	—	—	5	6	—	—	—
	—	1	13	Cr 4 ♀ (1)2/15								
37	—	—	25	Cr 5 ♂ 266	—	—	—	2	—[5]	—	—	—
	—	—	1	Cr 4 ♀ 19								
37A	—	47	59	Cr 5 ♀ (3)(9)750/152/252	—	—	—	3	5	—	—	—
	—	47	57	Cr 4 ♂ (2)(7)750/152/266								
37B	—	20	38	Cr 5 ♀ (1)(1)1000/263/(2)55/(4)/153/154	—	2	—	3	—	1	—	—
	—	23	71	Cr 4 ♂ (2)(2)450/6/408								
37C	38, 59, and 25[6]	28 and 38	59A	Cr 5 ♂[7]	—	—	—	—	—	19[10]	—	—
	71 and 66A[8]	74	19A	Cr 4 ♀[9]								

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[1] Seven of these carried the self "ghost" pattern in their coats; and probably others carried some pattern too.

[2] One of these parents carried albinism recessive, for experiment 31 shows that some of their offspring carried albinism, and experiment 56 that some of them were pure.

[3] Experiment 37B shows that this individual carried albinism recessive.

[4] One of the four albinoes carried the piebald "ghost" pattern in its coat.

[5] The remainder of this litter was probably eaten.

[6] For G–G–G–G–P, see experiment 47.

[7] The number is: (3)(7)(1)1000/263/(2)55/(4)153/154/(1)(5)(9)750/152/252/(1)(1)1000/263/(2)55/(4)153/154.

[8] For G–G–G–G–P, see experiments 5, 23, and 48.

[9] The number is: (1)(2)(5)52/53/(3)(2)450/(6)408/(3)(5)52/53(3)(2)450/6/408. This albino carried the self "ghost" pattern in its coat. Hence the self-pattern in its offspring.

[10] There were four others, pigmented and self-pattern, but they died too early to determine whether they were Cr 6 "*a*," Cr 6 "*b*," or Cr 7.

the expectation is 2 Cr 4+1 Cr 5+1 Cr 6 *b*. The result (experiment 35) is 8 Cr 4+3 Cr 5+2 Cr 6 *b*.

If the albino parent be cgBSP and the Cr 5 parent is homozygous, the expectation is that the offspring shall be all of the "Irish" *b* form. The result (experiment 37C) is nineteen "Irish" *b* individuals.

If the albino* is cGgBSP and the piebald carries c recessive, the expectation is

2 Cr 4+1 Cr 2+1 Cr 6 *b*;
the result is 3 „ +2 „ +1 „ (experiment 37B.)

A sister of this albino parent (experiment 37B) was also mated with a black piebald (experiment 34). No grey individuals appeared in her offspring of eight. This, of course, is evidence that segregation of characters, *i.e.* blackness and greyness, occurred.

(3) *Cr* 5 × Cr 5 = *Black Piebald* × *Black Piebald.*

(a) *Heterozygous Cr* 5 × *Heterozygous Cr* 5.

Experiments 25, 27, *and* 28A.

The heterozygous Cr 5 carries c. The expectation is 1 Cr 4 : 3 Cr 5. The actual result is 16 Cr 4+34 Cr 5.

If we add the results of other observers, the figures stand thus:—

† Crampe	12 Cr 4+45 Cr 5
‡ Doncaster	4 „ + 9 „
Mudge	16 „ +34 „
Total	32 „ +88 „ = 1 : 3 nearly.

With this total of 120 the predicted proportions are 30 : 90.

(b) *Heterozygous Piebald* × *Homozygous Piebald.*

Experiments 26 *and* 28.

The expectation in this case is that all the offspring will be piebald. The result is 11 piebalds. The further expectation is that some of the piebald offspring will be homozygous and some heterozygous. Experiments 31 and 56§ show respectively that the offspring of mating 26 was: one of them heterozygous and another homozygous.

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* The previous behaviour of this albino is worth noticing (see experiment 45, first paper).

† 'Land. Jahr.,' vol. 14, 1885, pp. 596 and 597.

‡ 'Camb. Phil. Soc. Proc.,' vol. 13, 1906, p. 223.

§ See first paper.

Summary.

These experiments show that albinoes breed true to albinism, whether their ancestry is pigmented or not. They further show that, though externally albinoes may appear to be identical with regard to their coat characters, in reality they may be different. This fact has been previously known for both animals and plants, but it has hitherto been elucidated by means of breeding tests alone. These experiments add an ocular demonstration of the actual presence of the coat-pattern in albinoes. The interpretation placed upon the coat appearances in these albinoes is corroborated by the breeding results.

It is further shown that when a piebald black rat (Cr 5) is mated with a similar one, two classes of offspring may be obtained. One of these contains all black piebalds and the other a mixture of black piebalds and albinoes in nearly equal numbers.

When a piebald black rat is mated with an albino (=Cr. 5 × Cr 4), it may be said that, so far as these experiments have gone, five different results will be obtained. They may be stated as follows : (1) The offspring are all black piebalds. (2) They may be a mixture of black piebalds and albinoes. (3) They may be all "Irish" forms (=a black self-coloured form). (4) They may be a mixture of albinoes, black piebalds, and "Irish." (5) They may contain albinoes, "Irish," and a grey form (=Cr 2). It can be shown that the divergency of the results obtained when two individuals apparently similar are mated is due to the gametic nature of the albino employed.

Have Trypanosomes an Ultra-microscopical Stage in their Life-history?

By Colonel David Bruce, C.B., F.R.S., Army Medical Service, and Captain H. R. Bateman, Royal Army Medical Corps.

(Received June 6,—Read June 25, 1908.)

By an ultra-microscopical stage in the development of a micro-organism is meant a stage in which the parasites are so small as to be invisible to the highest powers of the microscope, and to be capable of passing through the pores of a porcelain filter. For example, a drop of South African horse-sickness blood will give rise to the disease if injected under the skin of a healthy horse. If a similar drop is examined under the highest available powers of the microscope, nothing in the shape of a micro-organism can be seen. If this blood is filtered through a porcelain filter, the virus passes through, and the filtrate is found to be as infective as the original blood. Horse-sickness is therefore looked upon as a disease caused by an ultra-microscopical micro-organism.

For some time it has been reported by various workers that an ultra-microscopical stage exists among the trypanosomes. For example, Plimmer informs us that he found the filtered blood of nagana animals to be infective. Salvin Moore and Breinl write that the blood of animals suffering from *Trypanosoma gambiense* infection, although apparently containing no trypanosomes at all, and even if properly filtered, is still capable of infecting other animals into which it may be introduced. MacNeal also makes a similar statement in regard to *Trypanosoma lewisi.* He states that "in culture, on blood-agar, *T. lewisi* may give rise to much smaller forms, and that such cultures, after passage through a Berkefeld filter, still infect rats." Finally, it may be noted that the late Dr. Fritz Schaudinn, whose too early death we all lament, expressed the belief that trypanosomes may multiply by longitudinal division so rapidly as to become small enough to pass readily through a Chamberlain filter.

This subject is an important one, as the discovery of an ultra-microscopical stage in these trypanosomes might throw light on the causation of some diseases in which no parasite can be found. In kala-azar, for example, the intra-corporeal form is small, and the extra-corporeal a fairly large flagellated organism. Let us imagine that the Leishman body lying inside the splenic cells had still further subdivided and become ultra-microscopical, then we would have an invisible parasite causing a serious disease in man and developing outside the body into a clearly visible flagellate. It is evidently important, then, in studying such diseases as South African horse-sickness, to try the effect of planting out the blood on various media, and looking for a visible stage of development in different insect hosts.

The following experiments were made to test the truth of the statement that trypanosomes have this invisible stage. The filters used were Berkefeld's ordinary filters for laboratory use. They were tested before use and found to readily keep back *Micrococcus melitensis* from the filtrate. The apparatus was attached to a Sprengel's pump:—

To ascertain if the Filtered Blood of Rabbits suffering from Nagana is infective.

Experiment 23.

March 20, 1908.—A rabbit which was inoculated on the 7th February, 1908, with *Trypanosoma brucei*, was killed to-day in an advanced stage of nagana. On microscopical examination of the peripheral blood and blood from the heart, no trypanosomes were seen. Portions of the heart, lungs, liver, spleen, kidneys, and bone-marrow were pounded up in a mortar with 1 per cent. sodium citrate in normal saline. The resulting emulsion was then filtered through a Berkefeld filter, and 1 c.c. of the filtrate injected subcutaneously into each of two white rats.

April 30, 1908.—Both rats healthy. No trypanosomes have appeared in their blood.

Experiment 26.

April 24, 1908.—Rabbit inoculated with *T. brucei* on 26th March, 1908. Same procedure as in Experiment 23.

May 21, 1908.—Both rats healthy.

Experiment 35.

March 31, 1908.—Rabbit inoculated 7th February, 1908. Same procedure as in Experiment 23.

May 4, 1908.—Both rats healthy.

Experiment 51.

January 31, 1908.—Rabbit inoculated 10th January, 1908. Same procedure as in Experiment 23.

March 25, 1908.—Neither rat showed trypanosomes at any time in its blood.

Experiment 53.

February 3, 1908.—Rabbit inoculated 10th January, 1908.

March 30, 1908.—Result negative. Both rats healthy.

Conclusion.

From these five experiments it would appear that the blood or organs of rabbits suffering from nagana does not contain ultra-microscopical forms of *T. brucei.*

To ascertain if the Filtered Blood of White Rats suffering from Nagana is infective.

Experiment 40.

December 17, 1907.—A white rat, suffering from nagana, and whose blood was swarming with *T. brucei*, was killed to-day. The organs and bone-marrow were made into an emulsion with 1 per cent. sodium citrate in salt solution and filtered in the usual way. Half a cubic centimetre of the filtrate was then injected into the peritoneal cavity of a white rat.

March 16, 1908.—This rat has never shown trypanosomes in its blood.

Experiment 41.

December 17, 1907.—This rat was also injected with the same quantity of filtrate as in Experiment 40.

April 1, 1908.—Trypanosomes have never appeared in the blood.

Experiments 42 *and* 43.

December 24, 1907.—A nagana rat, whose blood was swarming with trypanosomes, was killed and the organs, etc., emulsified and filtered. One cubic centimetre of the filtrate was injected intra-peritoneally into two white rats.

March 30, 1908.—Both rats healthy.

Experiments 44, 45, 46, 47, 48, 49, and 50.

This procedure was repeated seven times in exactly the same way and always with a negative result.

Conclusion.

From these 11 experiments it would appear that the blood of nagana rats filtered through a Berkefeld filter is not infective.

To ascertain if the Filtered Blood of White Rats suffering from Nagana or Surra, and treated for various Periods with Antimony, is infective.

It was thought that the effect of treatment on animals suffering from nagana might lead to the development of small resting forms of the *Trypanosoma brucei* which might be capable of passing through a Berkefeld filter. The effect of certain drugs on animals suffering from nagana is marvellous. The blood may be swarming with trypanosomes, yet within an hour of the injection not a single one can be seen. They may remain out of the blood for weeks or months, in some out-of-the-way place, and, perhaps, in some resistant form.

Experiment 28.

March 26, 1908.—A white rat, whose blood was swarming with *T. brucei*, was treated with $\frac{1}{2}$ c.c. of a $\frac{1}{4}$-per-cent. solution of sodium antimonyl tartrate. The rat died half an hour after receiving this dose. The organs and bone-marrow were emulsified and filtered in the usual way, and 1 c.c. of the filtrate injected subcutaneously into a white rat.

May 7, 1908.—Trypanosomes have not appeared in the blood.

Experiment 29.

March 27, 1908.—A white rat, whose blood was swarming with *T. evansi*, was injected with ½ c.c. of a ¼-per-cent. solution of sodium antimonyl tartrate. This rat died half an hour after receiving the dose. Emulsion of the organs and bone-marrow made and filtered in the usual way, and 1 c.c. of the filtrate injected into two white rats.

April 29, 1908.—Both rats remain well.

Experiment 33.

March 30, 1908.—A white rat, whose blood was swarming with *T. evansi*, was injected subcutaneously with ½ c.c. of a ¼-per-cent. solution of sodium antimonyl tartrate. This treatment was continued for a month, the animal receiving in all 11 doses.

May 4, 1908.—Rat killed and its organs and blood emulsified and filtered. Half a cubic centimetre of the filtrate was injected intra-peritoneally into two rats.

June 4, 1908.—Both rats healthy.

Experiment 66.

March 8, 1908.—A white rat, whose blood was swarming with *T. brucei*, was injected with two drops of a 1-per-cent. solution of sodium antimonyl tartrate. This treatment was continued for a month, the animal receiving in all eight doses.

April 10, 1908.—Rat killed and its organs emulsified and filtered in the usual way. One cubic centimetre of the filtrate injected into two rats.

May 11, 1908.—Both rats healthy.

Experiment 19.

March 8, 1908.—A nagana rat, whose blood was swarming with *T. brucei*, was injected on the third day of disease with 2 minims of a 1-per-cent. solution of sodium antimonyl tartrate.

March 9, 1908.—Repeated injection. A few trypanosomes in blood.

March 11, 1908.—Repeated injection. A few trypanosomes in blood.

March 13, 1908.—No trypanosomes in blood.

March 18, 1908.—Blood swarming with trypanosomes. Injected ½ c.c. of a ¼-per-cent. solution of sodium antimonyl tartrate. Rat died five minutes later. Organs emulsified and filtered and ½ c.c. of the filtrate injected into a white rat.

March 26, 1908.—This rat's blood is found to be swarming with trypanosomes. It is evident that something has passed through the filter capable of infecting a rat with nagana ; but it is possible that the filter has become defective on account of wear. It was tried again with a cultivation of *Micrococcus melitensis* in broth and failed to keep back the micrococci from the filtrate. It was, therefore, concluded that the filter was defective, and this experiment null and void.

Conclusion.

From these experiments it may be concluded that the blood of white rats suffering from nagana and treated for varying times with antimony salts does not contain ultra-microscopical forms of *T. brucei*.

To ascertain if the Cultivation of Trypanosomes on Blood-agar will give rise to Ultra-microscopical Forms which are capable of passing through a Berkefeld Filter.

Experiment 24.—*White Rats.*

March 20, 1908.—The water of condensation from six flasks of blood-agar, upon which *T. lewisi* had been planted out for 18 days, was to-day filtered through a Berkefeld filter, and ½ c.c. of the filtrate injected into two white rats.

April 28, 1908.—These rats have remained in good health and no trypanosomes have appeared in their blood.

Experiment 37.—*White Rats.*

April 1, 1908.—A test-tube of blood-agar (2—1), which contained a luxuriant growth of *T. lewisi*, was shaken up with 25 c.c. of normal salt solution, and the resulting emulsion filtered through a Berkefeld filter. The filtrate was then injected into the peritoneal cavity of three white rats.

April 30, 1908.—All three rats remained well, and trypanosomes never appeared at any time in their blood.

Experiment 36.—*White Rat* (*Control*).

March 31, 1908.—To ascertain if the culture used in Experiment 37 was virulent, three drops of the condensation fluid were injected into a small white rat.

April 6, 1908.—*T. lewisi* appeared in the blood of this rat.

Experiment 64.—*White Rats.*

April 9, 1908.—A blood-agar tube containing a growth of *T. lewisi*, first generation, 46th day of growth, was shaken up with normal saline and filtered in the usual way. The filtrate was injected intra-peritoneally into two rats.

May 11, 1908.—Both rats well. Trypanosomes have never appeared in their blood.

Experiment 98.—*White Rats.*

May 7, 1908.—Two blood-agar tubes, 27th day of growth. Same procedure as in Experiment 64.

June 2, 1908.—Both rats healthy.

Experiment 97.—*White Rat* (*Control*).

May 7, 1908.—To ascertain if the culture used in Experiment 98 was infective, ½ c.c. of the condensation fluid was injected intra-peritoneally.

May 18, 1908.—*T. lewisi* in the blood.

Conclusion.

From these experiments it may be concluded that cultures of *T. lewisi* on blood-agar do not give rise to ultra-microscopical forms which are capable of passing through a Berkefeld filter.

The final conclusion arrived at is that neither *T. brucei* nor *evansi* develop in the body of animal forms so small as to be capable of passing through the pores of a Berkefeld filter, and that in cultures of *T. lewisi* on blood-agar such small forms are also absent.

Diphtheria Antitoxin.

By John Mellanby, M.D., George Henry Lewes Student.

(Communicated by Professor J. N. Langley, F.R.S. Received June 3,—Read June 25, 1908.)

(From the Wellcome Physiological Research Laboratory and the Physiological Laboratory, Cambridge.)

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HISTORICAL.

Many experimenters have endeavoured to isolate diphtheria antitoxin from fluids containing it.

In 1893 Brieger and Ehrlich (1) investigated the properties of milk obtained from animals which had been immunised against diphtheria toxin. This milk contained an appreciable amount of antitoxin which could be precipitated between 27- and 38-per-cent. ammonium sulphate. By this method they were able to increase the antitoxic value of their fluid about five hundred times. But in milk only two proteins are present which differ widely in their physical properties—caseinogen and lact albumin—and their results could not be extended to horse serum, which is the main source of diphtheria antitoxin. Their experiments did not show that antitoxin is not a protein.

Since horse serum is the main source of diphtheria antitoxin, experimental work has been done chiefly on this fluid.

Aronson (2) stated that globulin precipitated from antitoxic serum by dialysis contained antitoxin. But Dieudonné showed that globulin precipitated by carbon dioxide from antitoxic serum did not possess this property. The protein precipitate obtained by both these workers corresponded to the globulin of serum as originally defined. Dieudonné further showed that if Hammarsten's extended definition of globulin were adopted, then diphtheria antitoxin was a globulin, since it could be precipitated from serum by saturation with magnesium sulphate.

Brodie (3) found that the same result could be obtained by half saturation with ammonium sulphate.

Belfanti and Carboni confirmed the results of Brodie and Dieudonné.

Freund and Sternberg (4) attempted the isolation of diphtheria antitoxin from serum by using 1·6-per-cent. potash alum. They stated that the filtrate obtained from serum after adding this salt, when treated with ammonium sulphate, gave a precipitate which contained the greater part of the antitoxin associated with only a small quantity of the original protein of serum.

Pick (5) attempted to differentiate the antitoxic portion of serum by fractional precipitation with ammonium sulphate.

Pröscher first digested antitoxic serum with trypsin and then fractionated the resultant liquid by means of ammonium sulphate. He stated that in this way he obtained a solution containing all the antitoxin free from protein.

THE RELATION OF DIPHTHERIA ANTITOXIN TO THE NORMAL PROTEINS OF HORSE SERUM.*

Three proteins possessing well-defined physical properties may be separated from horse serum. These proteins are present in serum in the following quantities:—

(*a*)	Globulin	About 3 per cent.
(*b*)	Albumin (α)	„ 85 „
(*c*)	Albumin (β)	„ 12 „

In the investigation of the properties of diphtheria antitoxin the first question to settle was whether it belonged to the protein groups (*a*), (*b*), or (*c*).

The protein (*a*)—globulin—was obtained by precipitating antitoxic serum with 10 volumes of water and neutralising with acetic acid. The precipitate was adequately washed by decantation and filtration. This precipitate when dissolved in sodium chloride did not possess antitoxic properties. It may therefore be concluded that diphtheria antitoxin is not a globulin as rigidly defined. This result confirms the work of Dieudonné and Belfanti and Carboni.

The protein (*c*)—albumin (β)—is probably that protein of serum which can be crystallised by means of ammonium sulphate and sulphuric acid. Crystalline albumin was prepared from antitoxic horse serum by this method.

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* The experiments detailed in the following pages are based upon results previously described in two papers: "The Physical Properties of Horse Serum," 'Journ. Physiol.,' vol. 35, p. 473, 1907, and "The Precipitation of the Proteins of Horse Serum," 'Journ. Physiol.,' vol. 36, p. 288, 1907.

The crystals obtained when dissolved in water did not possess antitoxic properties.

It is evident, therefore, that diphtheria antitoxin belongs to or is associated with that great group of albumins which compose about 85 per cent. of the total protein of serum. For this class of albumin no method of physical differentiation has been obtained. It was interesting, therefore, to determine whether a delicate biochemical test such as is involved in the standardisation of diphtheria antitoxin would permit of a further differentiation.

The Freezing of Serum.

Serum containing 400 units per cubic centimetre of diphtheria antitoxin was frozen in a long cylinder and allowed to melt gradually. The resulting liquid was syphoned off in a series of layers. Antitoxin tests were made on some of the fractions and the relation of the antitoxic value to the percentage of protein determined. The following results were obtained :—

	Amount of solid in 100 c.c.	Antitoxic value.
	1·52 grammes	50+
	4·77 „	200+
	9·06 „	350+
	13·19 „	450+
	17·08 „	700+
Original serum ...	9·4 „	400

The + sign above indicates that the animals survived the tests without any serious symptoms. The results are conclusive,—the concentration of antitoxin follows the concentration of the protein on approximately parallel lines.

The Heating of Serum.

The relation of diphtheria antitoxin to the proteins of serum coagulable by heat was determined.

Serum containing 400 units of antitoxin per cubic centimetre was coagulated at a series of temperatures—the coagulum at each temperature being filtered off before further heating.

The following results were obtained :—

	Solids in each 100 c.c.	Antitoxic value.
Original serum	9·4 grammes	400
Filtrate after heating to 66° C.	9·27 „	400
„ „ 77° C.	3·04 „	50
„ „ 80° C.	2·6 „	50

Thus heating to 66° C. coagulated a little fibrinogen, but destroyed no antitoxin; but heating to 77° C. removed the greater part of the coagulable protein and also the greater part of the antitoxin.

The fact that diphtheria antitoxin can be heated up to 66° C. without destruction is interesting. Ferments and complements are stated to be destroyed when heated to 56° C. The greater stability of diphtheria antitoxin to heat differentiates it from these classes of bodies.

The Electrolysis of Antitoxic Serum.

When a constant current is passed into serum through zinc sulphate electrodes, a mass of insoluble protein accumulates at the anode and water comes out at the cathode, the relation between the water and insoluble protein being such that the concentration of solids in the electrolysed serum keeps constant. The influence of this procedure on antitoxic serum was determined.

A constant current at a pressure of 100 volts was passed into antitoxic serum through zinc sulphate electrodes for three hours. A considerable mass of insoluble protein accumulated at the anode and an equivalent quantity of water passed out at the cathode. At the end of this time the antitoxic value of the remaining serum was determined. This value was found to have remained constant; the antitoxin had neither accumulated nor diminished in the remaining serum.

This fact is capable of only one explanation—that diphtheria antitoxin possesses the same properties as the normal proteins of serum when tested by means of an electric current passed into it through zinc sulphate electrodes.

The protein mass which accumulated at the anode was also tested for antitoxin. This precipitate consists of a zinc sulphate compound of protein. The precipitate was dissolved in a minimal quantity of sodium hydroxide, and its antitoxic value determined. It was found to have an antitoxic value proportional to the amount of protein dissolved. This fact is interesting—the combination of the protein with zinc sulphate, although causing an insoluble compound to be formed, had not produced any chemical change. The resulting precipitate possessed the antitoxin value of the original protein when adequately dissolved.

The Precipitation of Diphtheria Antitoxin.

(A) *By Alcohol.*—The alcohol precipitation limits of horse serum containing 400 units of antitoxin in each cubic centimetre were determined. The following results were obtained:—

Percentage of alcohol.	Percentage of protein precipitated.
12·5	3·6
25·0	7·6
37·5	48·7
50·0	97
75·0	99

The greater bulk of the protein is seen to be precipitated between 25 and 50 per cent. alcohol.

Antitoxin tests were made on the filtrate after precipitating with definite percentages of alcohol. It may be stated that alcohol in the dilutions used does not destroy toxin. From the antitoxin tests it was found that no antitoxin was precipitated by 25 per cent. alcohol; 25 per cent. was precipitated by 37·5 per cent. alcohol; 30 per cent. by 40 per cent. alcohol; and 66 per cent. by 44 per cent. alcohol. From these figures it is legitimate to assume that antitoxin has the same precipitation limits as the general mass of protein of the particular serum used.

From the antitoxin tests on the alcohol precipitation of other sera it appeared that the greater part of the antitoxin was precipitated between 35 and 45 per cent. alcohol.

A point of some importance was to see whether the presence of antitoxin in serum had any general influence on the form of the alcohol precipitation curve. The quantitative precipitations of four sera of different antitoxin values were therefore determined.

Serum.	Antitoxin value per c.c.	Total solids in 100 c.c.
A	150	9·6 grammes
B	250	11·0 „
C	600	9·2 „
D	800	9·7 „

Percentage of alcohol.	Weight of protein precipitated from 100 c.c. of serum.			
	A.	B.	C.	D.
	grammes.	grammes.	grammes.	grammes.
30	1·7	1·1	—	—
33	2·53	2·0	2·0	2·0
36	2·86	3·4	3·1	3·3
39	3·8	4·6	4·1	4·7
42	4·83	6·0	5·2	5·7
45	6·7	8·3	6·3	6·9
48	7·96	9·92	7·3	8·0
50	8·52	—	7·7	8·3

There is some evidence from the form of the curves (which may be drawn from the above figures) that the presence of diphtheria antitoxin does affect the precipitation values. Thus the Curve A is slightly concave to the left, and Curve D is slightly convex to the left. Curves B and C show features intermediate between A and D. The form of that part of the precipitation curve is affected in which the antitoxin is precipitated, and it may be seen that the degree of variation is roughly proportional to the amount of antitoxin present.

From this we may assume that although diphtheria antitoxin has the same alcohol precipitation properties as the great bulk of the proteins of normal serum, yet it is something added, and not merely a normal protein with an antitoxic group attached to it.

Alcohol as a Protein Coagulant.

An attempt was made to differentiate diphtheria antitoxin from the normal proteins of serum by means of alcohol as a protein coagulant.

In the case of diphtheria antitoxin it was found that although alcohol added to serum did not affect its antitoxic value so long as no protein was precipitated, yet the rate at which precipitated antitoxin was destroyed by alcohol was the same as that at which the normal proteins of serum were coagulated. Therefore, so far as the coagulating action of alcohol was concerned, there was no evidence of any physical differentiation of diphtheria antitoxin from the proteins of normal serum.

Precipitation by Alcohol below the Critical Point.

The above results were obtained by the action of alcohol on diphtheria antitoxic serum at temperatures above the critical point.

The alcohol precipitation limits of diphtheria antitoxin from serum were obtained at temperatures below the critical point. As a result of a series of alcohol precipitation experiments at 2° C., it was found that the greater part of the antitoxin was precipitated with other proteins of serum between 16 and 28 per cent. alcohol. These antitoxin limits, so far as the quantity of precipitated protein is concerned, are practically the same as those obtained at temperatures above the critical point. The only difference between precipitation above and below the critical point was that in the former case a portion of the antitoxin was lost owing to the coagulating power of the alcohol, whilst in the latter case the total protein precipitate was soluble, and the whole of the antitoxin could be recovered.

(B) *Ammonium Sulphate.*—Brodie precipitated the antitoxin from horse serum in four parts by the gradual addition of ammonium sulphate to half saturation. He found no accumulation of antitoxin in any one fraction of protein precipitated, the result indicating that the antitoxin was not carried down in a purely mechanical way.

The limits for the precipitation of antitoxin by ammonium sulphate were determined. It was found that the initial precipitation of the antitoxin was practically coincident with the first precipitation of the proteins of serum, and was complete only after about 22 per cent. of ammonium sulphate had been added. If these points be examined on the ammonium sulphate precipitation curves, it will be found that they are practically coincident with the alcohol precipitation limits.

(C) *By Neutral Salts in the Presence of Acids.*—The precipitation of antitoxin by salts in the presence of acids was examined; 5 per cent. acetic acid and 0·25 per cent. hydrochloric acid were found to have no deleterious influence on diphtheria antitoxin at room temperatures. Controls showed that these acids in dilutions corresponding to the antitoxic value of the serum used did not destroy toxin. The precipitation of antitoxin by sodium chloride and 0·25 per cent. hydrochloric acid started when about 10 per cent. of the total protein of the serum was precipitated, *i.e.*, the first 10 per cent. of protein precipitate contained only a minimal amount of antitoxin. During the rest of the protein precipitation the antitoxin came down in a quantity proportional to the amount of protein precipitate produced. There was no indication of any differentiation of the antitoxin from the great bulk of the proteid precipitate.

(D) *The Mechanical Precipitation of Diphtheria Antitoxin from Serum.*—It has been stated above that the temperature at which antitoxin is destroyed differentiates it from ferments.

A few experiments were made to determine whether it possessed the property common to ferments of mechanical precipitation. Various flocculent substances precipitated in serum did not diminish its antitoxic properties. This absence of mechanical precipitation might have been due to the simple nature of the precipitants, *e.g.*, calcium phosphate and kieselguhr. A solution of colloidal ferric phosphate was therefore added to antitoxic serum. The colloidal ferric phosphate was precipitated by the ionised salts present in the serum. After it had settled to the bottom of the flask as a gelatinous mass, the antitoxic value of the remaining serum was determined. There was no diminution in its antitoxic properties. From these experiments it may be concluded that diphtheria antitoxin does not possess the property of associating itself with substances precipitated in a solution of it.

The Action of Ferments on Diphtheria Antitoxin.

0·2 per cent. hydrochloric acid has been stated above to have no destructive action on diphtheria antitoxin. But when serum is acidified with 0·2 per cent. hydrochloric acid and a quantity of pepsin is added, it is found that the antitoxic value diminishes at a rapid rate. This destruction of diphtheria antitoxin by pepsin *in vitro* is in line with the inability to immunise animals against a subcutaneous injection of diphtheria toxin by feeding with antitoxic serum. Trypsin also destroys diphtheria antitoxin, but much more slowly than pepsin.

With regard to the statement of Pröscher concerning the preparation of protein-free antitoxin by digesting antitoxic serum with trypsin, the following experiment is of interest.

400 c.c. of trypsin (Liquor pancreaticus Benger) were added to 2000 c.c. of serum and incubated at 37° C. After four and nine days respectively the quantitative precipitation of this digested mixture by ammonium sulphate was determined. The following results were obtained:—

After four days the amount of solid not precipitated by saturation with ammonium sulphate was 10 per cent. of the total solid; after nine days the quantity of solid not precipitated had increased to 24 per cent. of the total solid. The 14 per cent. increase represented digested protein.

The quantitative results for the precipitation of the proteins of serum after four and nine days' tryptic digestion were determined.

Percentage of Am_2SO_4 (solid).	Percentage of protein not precipitated.	
	After 4 days.	After 9 days.
18·75	82·4	98·2
20·0	74·5	91·6
21·25	—	77·0
22·5	55·8	70·0
25·0	42·0	56·2
27·5	34·0	46·6
30·0	26·6	40·7
35·0	20·0	—
40·0	—	31·7

There are two results to be noted from these figures:—

(1) The action of trypsin on the proteins of serum is slow, but progressive.

(2) The diminution of the amount of protein precipitated by ammonium sulphate after tryptic digestion is constant for all quantities of ammonium sulphate. Thus, in the above figures the average difference between the amount of protein left in solution by any amount of ammonium sulphate

after four and nine days' tryptic digestion is 15 per cent. The precipitation limits of the digested protein only are altered.

The first result indicates that the slow action of trypsin on the proteins of serum is due to the resistance of this type of protein to tryptic digestion rather than to the presence of an antibody to trypsin in the serum. The second result emphasises the need for quantitative experiment when precipitating digested mixture of protein with ammonium sulphate, more especially if conclusions as to the antitoxic value of the precipitate are required.

The rate of digestion of the protein as given by the above figures is approximately the rate at which the antitoxin is destroyed by trypsin.

Conclusion.

The results of all the above experiments on diphtheria antitoxin may be briefly stated :—

(*a*) Whenever the main protein of serum is precipitated, diphtheria antitoxin is precipitated.

(*b*) Whenever the main protein of serum is destroyed or coagulated, diphtheria antitoxin is destroyed.

These results have only one logical explanation—that diphtheria antitoxin is a protein.

There is a possibility that diphtheria antitoxin is a comparatively simple body, and is attached to the protein only by virtue of some chemical affinity. But in the above experimental work a large number of methods of analysis have been used, and it is reasonable to suppose that if the hypothesis above were correct, some indication would have been obtained of a splitting of the antitoxin from its accompanying protein. No such indication has been obtained. If we assume, then, that the diphtheria antitoxin is a protein, what hope is there of isolating it in a pure condition ? Serum doubtless contains a very large number of proteins which possess different physiological properties. But a living cell is required to appreciate these physiological differences. In laboratory work the only available forces are those of a physical and chemical nature. With these forces it is possible to differentiate the proteids of horse serum into three groups :—

(*a*) Globulins comprising about 3 per cent. of the total protein.

(*b*) Albumins which cannot be crystallised. These compose about 85 per cent. of the total proteins.

(*c*) Albumins which may be crystallised. These compose about 12 per cent. of the total protein.

Diphtheria antitoxin belongs to the class (*b*), composing about 85 per cent. of the total proteins of serum.

The similarity of diphtheria antitoxin to the main mass of the protein of serum raises the question as to the function of these proteins.

It is generally assumed that the main function of the proteins of serum is to nourish the tissues. From a general consideration this is improbable.

Foodstuffs are divided into three great classes:—proteins, fats, and carbohydrates. Sugar is present in blood to the extent of about 0·2 per cent., and usually only a trace of fat can be detected in it. It is improbable, therefore, that proteins to the extent of 10 per cent. are circulating in the blood to be used as food.

From a study of the processes involved in the coagulation of blood it is fairly clear that globulin originates from a splitting of the fibrinogen molecule into fibrin and globulin when coagulation occurs. Albumen (β) composing 12 per cent. of the total protein may possibly be regarded as concerned in the nutrition of the tissues. Since diphtheria antitoxin belongs to the protein group composing the remaining 85 per cent. of protein, it may be legitimate to assume that a function of these proteins is to protect the tissues from disease in addition to maintaining the equilibrium of electrolytes in the tissues, and holding carbon dioxide in the blood. The assumption of such a function would afford an explanation why large injections of normal horse serum after control diseases caused by bacterial infections.

THE RELATION BETWEEN THE ANTITOXIC POTENCY OF A SERUM AND THE AMOUNT OF PROTEIN CONTAINED IN IT.

The relation between the antitoxic power of a serum and the solids contained in it has been investigated by various workers.

Szontagh and Wellmann (6) found only a small increase in the total solid present in serum after immunisation against diphtheria toxin.

Hiss and Atkinson (7) found a rise of globulin content during the process of immunisation and a corresponding fall in the albumen content. The globulin was determined by precipitation with magnesium sulphate.

Ledingham (8), using half-saturation with ammonium sulphate as a precipitant, also found an increase of globulin content during immunisation.

The work of Hiss and Atkinson, and Ledingham, was based on the assumption that diphtheria antitoxin was a globulin as defined by its precipitation limits with ammonium sulphate and magnesium sulphate. But from a consideration of the influence of acids and alkalis on the precipitation of the proteins of serum by neutral salts, it is clear that their results could be adequately explained by an assumption of varying alkalinity of the serum during immunisation.

It has been shown in the previous pages that diphtheria antitoxin possesses the same physical properties as nine-tenths of the proteins of serum, and that this antitoxic protein is probably added to serum during the process of immunisation. The question arises as to whether the theory of the addition of antitoxic protein to the blood during the process of immunisation receives any support from a consideration of the total solids in the blood of a horse during its antitoxic life.

A detailed examination of the quantity of protein in the sera of various horses showed that there was no relation between the total proteins in the blood of a horse and its capacity to produce antitoxin. From the antitoxic point of view we may consider the proteins present in the serum of an animal as concerned in two great functions: (*a*) The maintenance of tissue equilibrium; (*b*) the protection of the tissues. Of these two divisions (*a*) is the more active component, and would be more evidently reflected in the amount of protein contained in the serum. The absence of any relation between the antitoxic producing powers of different animals and their general metabolism reflects the experience of individuals—that a strong physical condition does not imply a special capacity to resist disease.

But the question whether the percentage of solids in the blood of a horse at different times of its antitoxic life bears any relation to the quantity of antitoxin in it is of a different nature. In this case it appears reasonable to assume that, so long as we do not interfere with the general nutrition of the animal by poisoning with excess of toxin, the percentage of solid in the serum will increase with its antitoxic power. To test the accuracy of this assumption, the percentages of solid in the sera of a number of horses at different periods of their antitoxic life were determined.

In the following table (p. 410) the days are reckoned from the commencement of immunisation.

From the results we may deduce two generalisations:—

(*a*) That at the start of diphtheria toxin immunisation the percentage of solid increases with the antitoxic power.

(*b*) That after a time, depending on the particular horse, there is no connection between the percentage of solid in the serum and its antitoxic power. This stage is marked by a large decline in the antitoxic producing power of the horse.

These two generalisations are readily appreciated if we consider the protein in the serum of a horse to reflect the two functions stated above: (i) the maintenance of tissue equilibrium; (ii) the protection of the tissues. The

Days.	Antitoxic value.	Percentage of solids in serum.	Days.	Antitoxic value.	Percentage of solids in serum.
	Horse A.			Horse B.	
0	0	8·93	0	0	7·78
60	225	—	97	350	9·02
104	500	10·91	148	1000	10·54
146	675	11·98	222	450	9·54
190	575	12·10	266	250	10·6
235	800	11·35	313	500	10·4
254	450	9·88	355	363	10·3
302	700	11·53	395	250	10·1
348	700	11·52	443	200	10·77
397	800	12·11	506	200	10·76
460	575	12·0			
496	900	12·1		Horse C.	
533	500	11·76	0	0	8·2
574	800	10·75	54	250	8·95
624	250	11·18	99	660	9·6
700	333	10·81	143	333	9·04
750	363	10·85	192	363	9·43
810	250	11·88	241	571	9·65
			292	400	9·8
			341	500	9·4

decline of the antitoxin producing power implies the exhaustion of that mechanism by means of which antitoxin is produced. That this is not reflected in the amount of solid in the serum depends upon the fact that the exhaustion of this special mechanism does not imply a corresponding effect on the general nutrition of the tissues.

EHRLICH'S THEORY OF ANTITOXIN PRODUCTION.

The salient point in the production of diphtheria antitoxin is that toxin in increasing doses is injected under the skin of a suitable animal, and, after a variable time, antitoxin appears in the blood.

The explanation of these phenomena which has been most widely accepted is that of Ehrlich. This theory may be stated in general terms as follows:—

The toxin combines with the side chain of a cell and so upsets the equilibrium of that cell. By virtue of its protective mechanism the cell manufactures a series of new side chains similar to that attacked; but these side chains are manufactured in greater quantity than is required to neutralise the toxin, and those in excess are cast off and appear in the blood as antitoxin.

This theory demands—

(*a*) That the tissues which are concerned in the production of antitoxin are those tissues which the toxin attacks.

(*b*) That antitoxin is produced in tissues remote from the seat of inoculation.

(*a*) From a study of the lesions produced by diphtheria toxin, it is clear that the main tissues attacked by this poison are the heart and nervous system. Therefore, on Ehrlich's theory, these tissues are mainly concerned in the production of antitoxin. From a general consideration of the metabolism of an animal it is improbable that two master tissues as the heart and nervous system are responsible for the protection of it. A horse had been immunised against diphtheria toxin for five years. At the end of this time it was killed, although it was suffering from no obvious disease. During its antitoxic life it had produced about 200 litres of antitoxic serum, containing, on an average, 500 units of antitoxin in each cubic centimetre, and had been injected with an appropriate amount of diphtheria toxin. A detailed *post-mortem* examination of the various organs was made. There were no marked pathological changes to be noted—the heart showed a little brown atrophy, but no changes were found in the nervous system. It is inconceivable that the heart and nervous system could have been attacked by such enormous quantities of toxin and have produced such a large amount of antitoxin without being more seriously affected.

A consideration of the amount of antitoxin produced by a horse after the injection of a definite quantity of diphtheria toxin is of interest. During a period of four weeks 800 prüfungs doses of toxin were subcutaneously injected into a horse. At the end of that time the blood contained 400 units of antitoxin per cubic centimetre. Suppose the horse contained 50 litres of blood. At the end of four weeks the blood of the animal contained $1000 \times 400 \times 50 = 2{,}000{,}000$ units of antitoxin, or 800 prüfungs doses of toxin produced 2,000,000 units of antitoxin; or each prüfungs dose of toxin produced 25,000 units of antitoxin.

If this be interpreted in terms of Ehrlich's hypothesis, it means that when the side chain of a cell is attacked by a molecule of toxin it manufactures more than 25,000 new side chains to protect itself. And if Ehrlich's hypothesis be correct, the cells which are concerned in this extraordinary production are contained in those tissues which perform the main functions of the body—namely, in the heart and nervous system.

(*b*) The production of antitoxin in a tissue remote from the site of injection of the toxin demands the assumption of other specialised mechanisms.

Let us consider the case of a horse towards the end of its immunisation period. At the last injection, probably, an amount of toxin containing 200 prüfungs doses is injected, and at this stage the blood of the animal contains 400 units of antitoxin in each cubic centimetre. This toxin, before it can produce any effect, must travel in the lymph stream to the thoracic duct, and so *viâ* the jugular vein into the general circulation. When once in the blood it will be ultimately carried into the neighbourhood of any tissue. But before it can attack a special tissue it must first pass through the capillary walls of the blood-vessels into the lymph bathing the cells of that tissue, and such a passage demands a specialised mechanism of chemiotaxis.

Further, from the union of toxin and antitoxin *in vitro*, it is probable that the toxin would unite with the antitoxin as soon as it got into the blood stream, and the hypothesis of the formation of antitoxin in a distant tissue would demand that this toxin-antitoxin compound should be broken up by the cell before it could stimulate the production of more side chains—in fact, that the formation of antitoxin by a cell should be of no use in protecting it from subsequent inoculations of toxin.

From a general consideration of diphtheria toxin immunisation it is probable that the leucocytes are the immediate agents concerned in the production of antitoxin. After a subcutaneous injection of diphtheria toxin into a horse there is usually produced at the site of inoculation a swelling of varying size. This swelling is of an œdematous character, and is crowded with finely granular oxyphil cells. The size of the swelling gives, to some extent, an indication of the degree of antitoxic reaction. The production of antitoxin by the leucocytes at the seat of inoculation would bring the mechanism involved into line with the facts observed by Metchnikow with pathological bacilli and the more recent work on opsonins. The antitoxin need not necessarily be produced at the seat of inoculation. After a leucocyte had ingested a toxin molecule the secretion of antitoxin by it would take place into any fluid in which it was present—the blood or lymph.

This hypothesis affords a ready explanation of poisoning by excess of toxin. If the leucocytes are able to take up the toxin, none of it is carried to distant tissues, such as the heart and nervous system, and no pathological effects are observed. But if the toxin is injected in too great a quantity to be taken up by the leucocytes, then the excess is carried by the blood to the tissues, and general toxæmia results. Again, Ehrlich's hypothesis offers no explanation why subcutaneous injection of toxin yields much better antitoxic results than injection into the blood stream direct. The theory that antitoxin is secreted by leucocytes which have ingested toxin molecules affords a ready explanation of these results.

SUMMARY.

All the properties of diphtheria antitoxic serum indicate that diphtheria antitoxin is a protein which possesses characters identical with albumin (α) of serum, *i.e.*, the protein forming 85 per cent. of the total quantity present.

The alcohol precipitation curves of antitoxic sera of varying strength

indicate that this protein is added to serum during immunisation, and that the antitoxic property is not due to the addition of a group possessing this character to a normal protein molecule. This conclusion also follows from a study of the relation between total solid and antitoxic strength of the serum of a horse during its antitoxic life.

It is suggested that one of the functions of the proteins composing the group albumin (α) is to protect the tissues against disease.

A theory is advanced that the production of diphtheria antitoxin is due to an active secretion by the leucocytes, this secretion being stimulated by the ingestion of toxin molecules.

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(1) Brieger and Ehrlich, 'Zeitschr. f. Hygiene,' vol. 12, p. 137.
(2) Aronson, 'Berl. klin. Wochenschr.,' vol. 26, p. 425.
(3) Brodie, 'Journ. of Pathol. and Bacteriol.,' vol. 4, p. 460.
(4) Freund and Sternberg, 'Zeitschr. f. Hyg.,' vol. 31, p. 429.
(5) Pick, 'Hofmeister's Beitr.,' vol. 1.
(6) Szontagh and Wellmann, 'Deutsche med. Wochenschr.,' vol. 24, p. 421.
(7) Hiss and Atkinson, 'Journ. of Exp. Med.,' vol. 5, p. 47.
(8) Ledingham, 'Journ. of Hygiene,' vol. 7, p. 65.

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(8) Ledingham, 'Journ. of Hygiene,' vol. 7, p. 65.

Croonian Lecture.—*The Principles of the Minute Structure of the Nervous System as revealed by Recent Investigations.*

By Professor Gustaf Retzius, For. Mem. R.S.

(Lecture delivered May 14,—MS. received June 15, 1908.)

When the flattering invitation to deliver this year's Croonian Lecture before your far-famed Royal Society reached me, I first of all felt considerable hesitation as to whether I should be able to discharge so honourable a task.

The very choice, out of the field of my investigations, of a subject which should be suitable for lecturing to you about, presented a very real difficulty. During the past 20 years I have been working principally in three departments of scientific research—*the Nervous System of Vertebrata and Invertebrata, Physical Anthropology in Sweden*, and *the Spermia of Animals of all Orders.*

By far the most interesting of these subjects is the first, dealing as it does with the chief organ in nature, that of the psychical functions. Upon this subject, of the minute structure of the nervous system, there has been a great deal of light thrown during the last two decades by the histological researches of a number of scientists, of whom may be specially mentioned the eminent Italian and Spanish neurologists Camillo Golgi and Ramôn Cajal.

The subject, however, has two real drawbacks: on the one hand it is exceedingly complicated, especially as several of its results are still under debate; and on the other it has already, in 1894, been treated of in a Croonian Lecture by Professor Cajal. Since his lecture, however, 14 years ago, the discoveries made in this department of science have been very numerous, many of them due to the researches of Cajal himself. At last I came to the conclusion that I might on this occasion continue and bring up to date the review of the subject which he then gave you.

In pursuing this intention I have, however, been obliged here and there to glance back at work done prior to 1894, and also to speak of some aspects of the subjects upon which my predecessor only touched very lightly. I conclude that it is in accordance with the idea of these lectures that the lecturer may also mention some of his own researches. And I hope that you will pardon me for expressing my opinion more definitely in certain particulars, seeing that I have arrived at a conviction of my own respecting them, which is based upon investigations which I have myself carried out. I regard this as unavoidable when the field of research in question consists of so much unbroken ground and such important problems are still not solved. It will all the same be necessary for me to limit my attention to a few of the most interesting chapters in this wonderful and fascinating department of biology.

The scientific investigation of the histology and physiology of the central nervous system, above all of the brain, is surely one of the most difficult problems presented to human intelligence to solve. With good reason Emil Dubois Reymond's famous ejaculation: "ignorabimus," may be applicable here.

"E pur si muove." How rapidly has our physiological knowledge of the localisation of the *motor* and *sensory centres* in the brain—since Fritsch and Hitzig first showed their existence by experimental proof—gone forwards step by step, owing principally to the brilliant discoveries of the English investigators Sir Victor Horsley, Schäfer, Beevor, Ferrier, Sherrington, and still others.

It is indeed true that the proverb, "There is nothing quite new under the sun," is not without an illustration here, too, for one cannot but be astounded to find that, as far back as the year 1744, the Swedish polyhistor and scientist, Emanuel Swedenborg, was able, in his famous work 'Œconomia Regni Animalis,' with his prophetic vision to set up as a goal for the science of physiology of the brain the following standard: "Experientiæ est et temporis, ut investigetur qui gyrus et qui serpens tumulus in cerebro hunc aut illum musculum ut correspondentem suum in corpore respiciat," and "Ergo inquirendum venit, qui tori corticei his aut illis musculis in corpore correspondent: quod fieri non potest nisi per experientiam in vivis animalibus, per punctiones, sectiones et compressiones plurium, perque inde in corporis musculis redundantes effectus." As we see, this is nothing short of a full programme in the experimental physiology of the brain which this marvellous man here lays before us, and we are yet again amazed to read his clearly worded statement, that the muscles of the lower extremities have their centre at the top of the cerebral cortex, the muscles of the abdomen and thorax in the central portions of the cerebrum, those of the head and face at the bottom, "nam videntur ordine inverso sibi correspondere." It has been my purpose in quoting these theses of Swedenborg's to point out that grand scientific discoveries, of which our own age is rightly proud, may have been not only vaguely guessed at, but actually set forth in clear and definite terms by one or another brilliant enquiring mind of an earlier age. The theses cited are drawn up with such precision by Swedenborg that they cannot possibly be based on divination only, but must rest upon a real grasp of natural phenomena as well as on actual experiments and dissecting work.

A more thorough knowledge of the minute structure of the brain and the whole nervous system was essential, if the physiology of those organs was to advance. To that end the perfecting of the microscope was a *conditio sine quâ non.* Earlier anatomists, *e.g.*, Leeuwenhoek and Malpighi, had paved the way, it is true, to our present results, but did not proceed far themselves. In accord with the last-named great Italian scientist (Malpighi), Emanuel Swedenborg, though, put forward a remarkable theory regarding the composition of the cerebral cortex, which he—in opposition to so many anatomists of that day—definitely declared to be the seat of the psychical phenomena.

Not until during the last century was it possible to get nearer the solution of this the most difficult problem of histology. It had by degrees been discovered that the nerve-tissues consist of nerve-cells in several different forms, and of nerve-fibres with and without myelin sheaths. It was also found out that the nerve-fibres are processes of the nerve-cells. The attempt was made to devise methods for tracing the nerve-fibres in their several courses, partly by hardening and staining them, partly by effecting their degeneration along certain paths, partly by studying the gradual development of their myelin sheaths in the embryo. By means of such researches scientists, before the close of the seventies, had arrived at the point of being able to establish theories concerning the minute construction of the nervous system in general. But, alas! how hypothetical, how uncertain these theories for the greater part were! I have still a clear remembrance of the hesitation and reluctance which I felt year after year, from 1877 onwards, in lecturing, as professor of histology, about this subject to my classes of students. I did not myself believe in a good deal of what I was constrained to teach them in accordance with the then accepted doctrines of science concerning the structure of the central nervous system. As a result of the investigations which I conducted in conjunction with Axel Key in the years 1869 to 1876, into the lymphatic spaces, and the structure of the brain, and the rest of the nervous system, I had arrived at the conviction that the abstract theories then held by men of science as to the construction of that system must be incorrect and were inconclusive.

It was at this period the Italian scientist Camillo Golgi made his invention for staining chrome-hardened nerve tissue with silver solution, by which the nerve-cells were selectively dyed brown. At first his communication, which was published in some smaller Italian journals with a very limited circulation in other countries, attracted very little notice, but on the appearance in 1885 of his great work, "Sulla Fina Anatomia degli Organi Centrali del Sistema Nervoso," men of science were surprised at this invention and its results.

Now, von Kölliker, myself, and others repeated this method, but, though we carefully followed Golgi's directions, the results were not really satisfactory.

I then began working by the new method of P. Ehrlich, which he published in 1886, and which consisted in staining living nerve tissue methylene blue.

Golgi's chrome-silver method had, however, in the inventor's own hands, yielded a series of fundamentally important results, which are already mentioned in the Croonian Lecture of Cajal. Of these the following may be specially mentioned here also. The occurrence in the central organ of two types of nerve cells, those whose axis-cylinder process does not branch and come to an end until after a long course, and those whose processes, after a short course, branch copiously, and come to an end in the central organ; further, the discovery of fine collateral branches emerging out of the axis-cylinder processes in the central organs; a more exact knowledge of the forms of the cells in the cortex of the cerebrum and the cerebellum, and more especially also in the gyrus hippocampi; the investigation of the different types and methods of ramification of the dendrites, and an enquiry into the structure of bulbi olfactorii. Golgi had become convinced that in the grey substance of the spinal cord there exists an exceedingly extensive and delicate network, which he considered was due to anastomosing of the collateral branches, and not, as Gerlach before supposed, to the anastomosing of the dendrites.

During the next following years, however, Golgi's method did not yield any very remarkable results; the scientists who used it did not obtain any real success with it.

Ehrlich's methylene method, on the other hand, was tried and valued in some places, especially in Kazan by Arnstein, Dogiel, and Smirnow, and in Stockholm by myself. In the higher animals, the vertebrata, the method could be well applied to the peripheral nervous system, but to the central one only with the utmost difficulty. I then determined to try and find some *lower* animals which admitted of having their whole nervous system, or, at any rate, the principal parts of it stained, so that by experimenting with them one might obtain a comprehensive idea of their entire construction. At last, in 1890, I found, with a certain modification, that the method gave excellent pictures in the ganglions of the ordinary crawfish (Astacus). A general survey was obtianed of their construction. One could clearly perceive that their so-called "Punktsubstanz" of Leydig was formed of the lateral twigs of the processes of the unipolar nerve-cells, which twigs could be traced in the substance in their most delicate ramifications, where they did *not* unite with each other, and on the whole did *not* form a *network* (reticulum), but a *twist-work* (plexus). One could also trace the stem-processes of the nerve-cells even in their various courses through the ganglions towards the periphery (fig. 1).

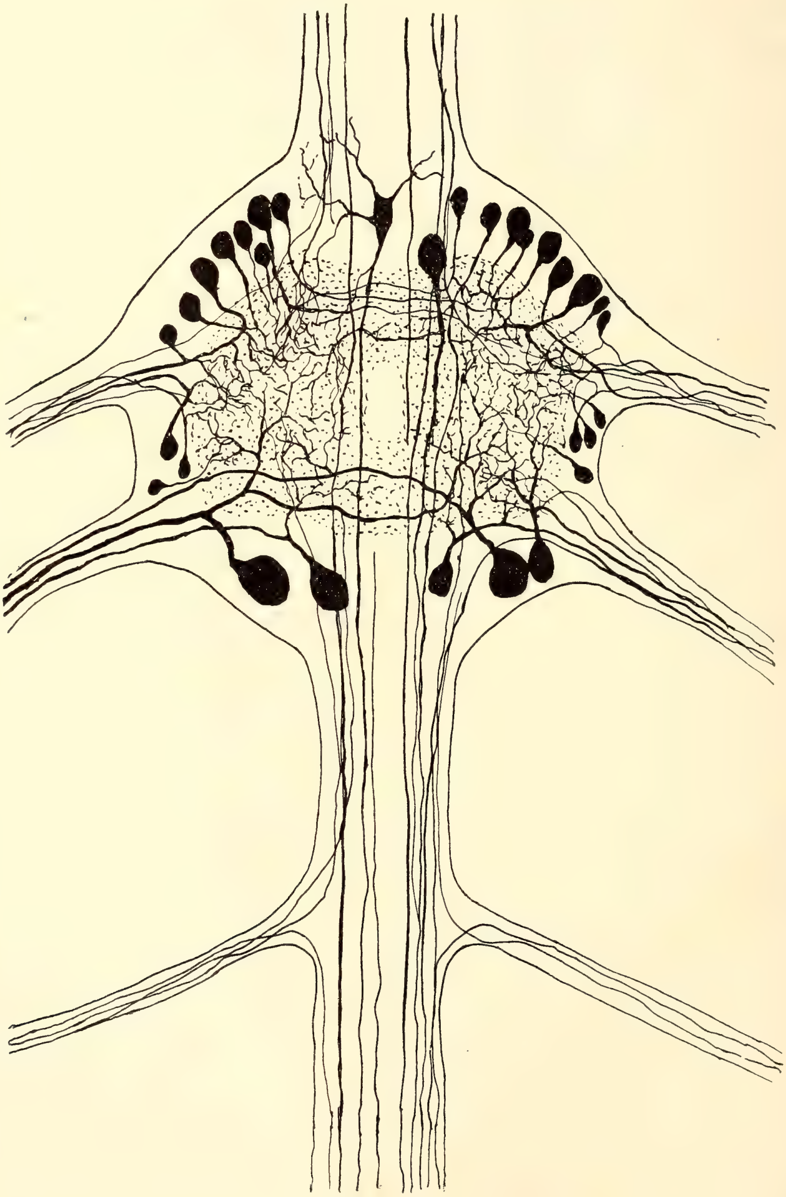


Fig. 1.—A Part of the Ventral Nervous Chain of the common Crawfish (Astacus), showing a ganglion with coloured (black) nerve-cells and their processes.

By similar researches on the spinal cords of Amphioxus and Myxine, I could also trace the condition of their central nerve-cells and the several paths of the cell processes. Still more beautiful and instructive pictures gave the nervous system of the Hirudines (fig. 2), where also Biedermann had studied them with success, and especially in that of a polychæt annelid (*Nereis diversicolor*).

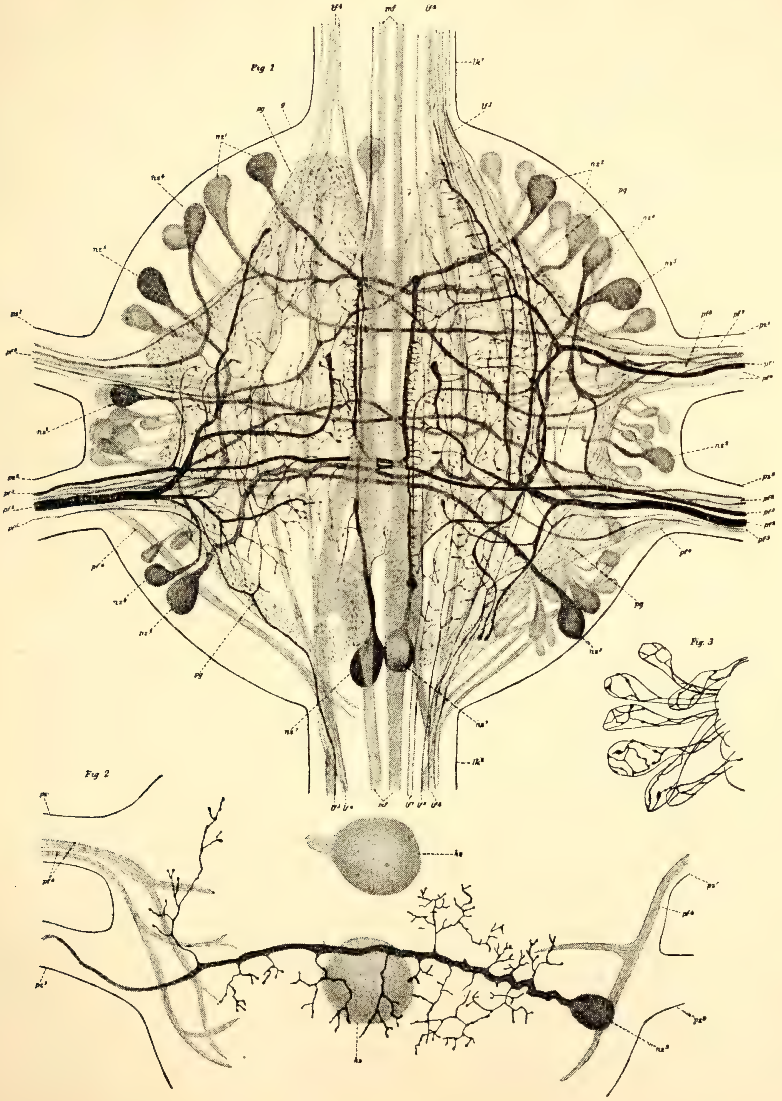


Fig. 2.—A Ganglion of the Ventral Nervous Chain of Hirudo, showing the black stained unipolar nerve-cells with their processes. The figure below shows a part of such a ganglion with a nerve-cell and its processes.

Now, in the years 1891 and 1892, the renowned Hungarian histologist Michael von Lenhossék, applying successfully the Golgi method in the common earth-worm, Lumbricus, discovered that the epithelium cells in the epidermis send fine fibres to the ganglions of the central nervous system, where they divide and terminate with free endings. These cells showed themselves very clearly to be a kind of sensory peripheral nerve-cells, which are in contact with the elements of the nervous central organ, and are capable of conveying to them impressions from the periphery of the body. This discovery of von Lenhossék was shortly after confirmed by myself and developed in conjunction with a closer investigation of the structure of the ganglions (figs. 3 and 4). I showed, by means of the methylene and chrome-silver methods, that similar cells exist in the epidermis of polychæts and molluscs, and also in the crustacea, though the cell body in these animals lies lower down under the epidermis (figs. 5 and 6).

Besides these sensory cells there exist also in the epidermis and the other epithelial tissues ramifications of free endings of tactile nerves, whose cells are to be found deeper.

I have mentioned these investigations in connection, particularly as they have for the most part been carried out by the methylene method. Now, however, a new era had commenced for Golgi's chrome-silver method, as applied to the central nervous system of the higher animals.

In and after 1888 Ramón Cajal, the great Spanish neurologist, published a series of works on the minute structure of the brain and spinal cord of vertebrate animals. By means of Golgi's method, which he improved and used in a masterly manner, Cajal succeeded in investigating the forms and the distribution of the nerve-cells and their processes in the different parts of the central nervous organ. We may date from this time almost a new epoch in our knowledge of the minute structure of the nervous system.

Cajal's first discoveries had an electrifying effect upon those who were working in the same field. For my part I shall never forget the overwhelming impression that the demonstration by Cajal, at the Berlin Anatomical Congress of 1889, of a large series of his preparations produced upon those of us who were specially interested in the subject. Albert von Kölliker and I were enchanted by the sight of the preparations which Cajal placed before us. Both he and I were converted, and we started home to begin working afresh with Golgi's method, which was not in great repute among other anatomists of that day. Kölliker, as well as von Lenhossék, working then in Kölliker's laboratory as his prosector, succeeded in applying Golgi's method, and published several excellent new researches. At the same time I, in Stockholm, and Van Gehuchten, in Louvain, were applying the same method, while

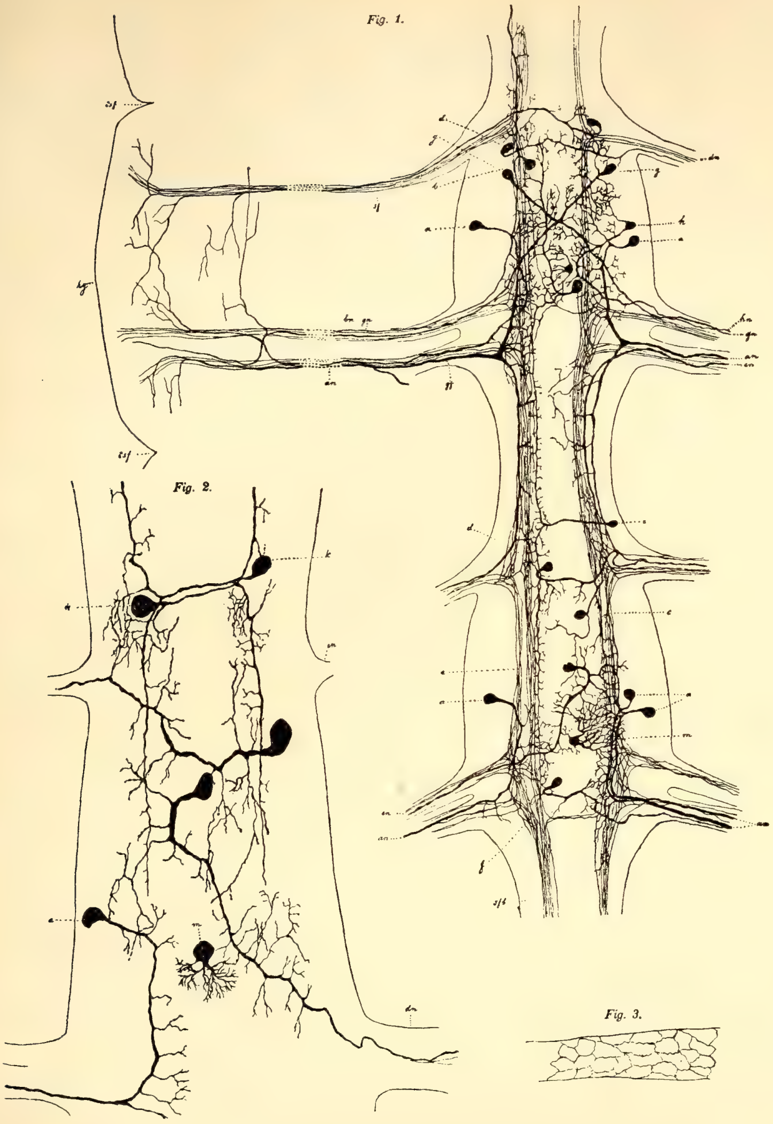


Fig. 1.

Fig. 2.

Fig. 3.

Fig. 3.—The Nervous System of Lumbricus. Parts of the ventral nervous chain of ganglions, with nerve-cells and their processes (nerve-fibres) stained black. 1. Two ganglia; at the upper the sensory nerve-bundles from the cells of Lenhossék are seen coming from the periphery and entering the ganglion, there dividing and terminating in free endings.

Cajal himself went on with one investigation after another, and Golgi and a couple of his pupils continued pursuing their various researches.

It may be said, nevertheless, that Cajal during this whole period was the most leading spirit in this research, so far as the central organ of the

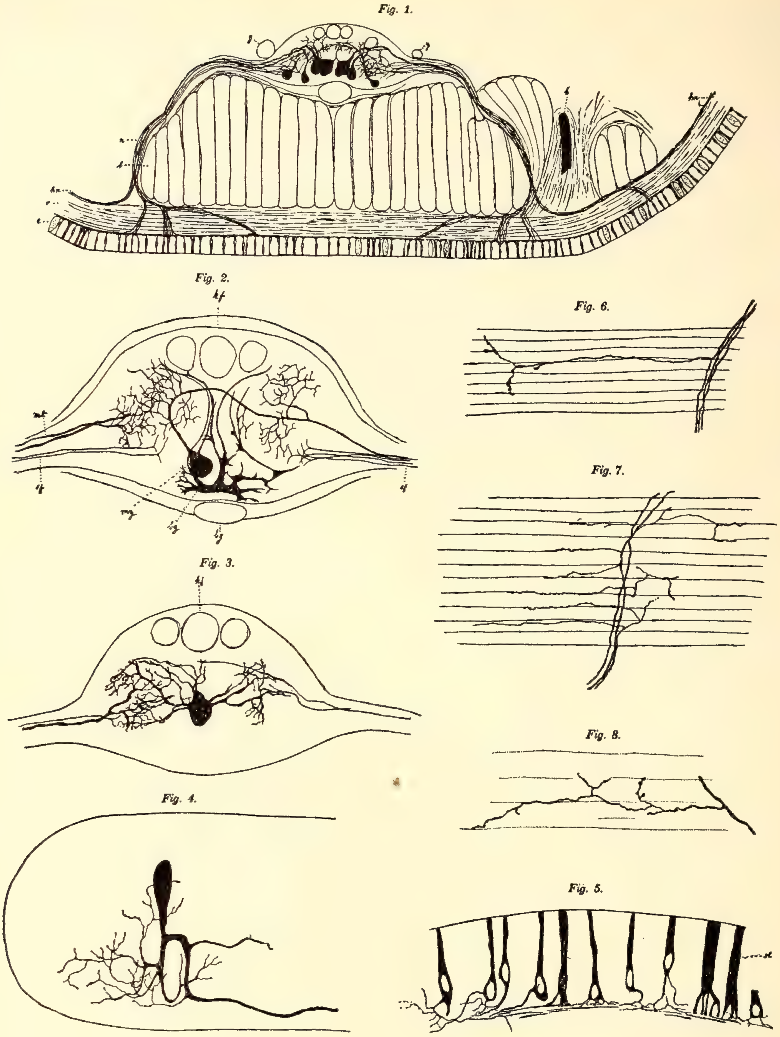


Fig. 4.—Nerves of Lumbricus, stained with the aid of the Golgi method. 1. A portion of a transverse section, with the skin and the cells of Lenhossék, with their central processes running to a ganglion. 2, 3, and 4. Transverse sections of ganglia, with nerve-cells and their processes, and also with some central fibres of the cells of Lenhossék. 5. A little portion of the skin, with the cells of Lenhossék. 6, 7, and 8. Endings of nerve-fibres on muscles.

vertebrate animals was concerned. Of Cajal's works there may here be cited in the first place those dealing with the spinal cord, the cortex of cerebrum and cerebellum, and several of the interior cerebral ganglia, the retina, the olfactory organ, and the peripheral ganglia (the sympathetic and spinal ganglia). In all these organs Cajal not only made important discoveries of

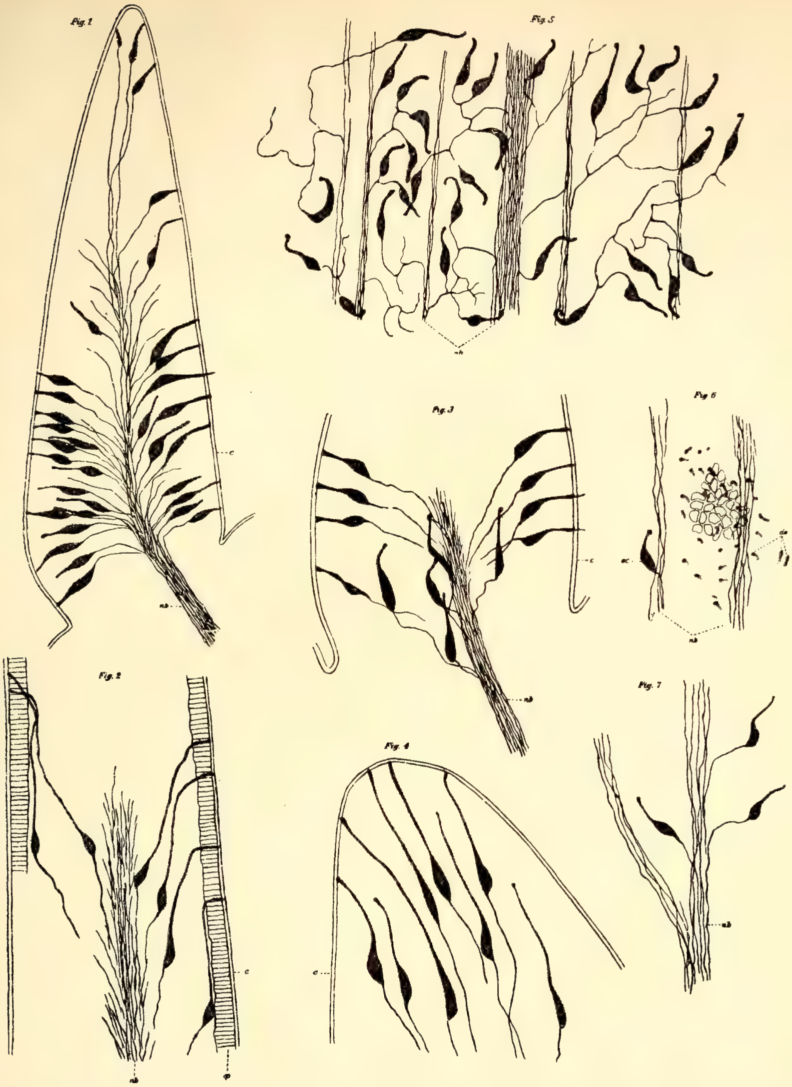


Fig. 5.—Portions of Antennæ, Parapodium, and Skin of *Nereis diversicolor*, with stained bipolar sensory cells and their processes.

new cell types and ramifications of cell processes, he also established the relations of the cells to one another in many respects. It would take too long to give a detailed account here of these researches, and the investigator himself in his Croonian Lecture to this distinguished Society, in 1894, described several of his most important discoveries in this branch of science.

Wilhelm His, of Leipzig, the eminent anatomist and histologist, had formed the conviction as long ago as in 1883, from his studies of the nervous system of the embryo, that the nerve-cells develop from the very first as organs

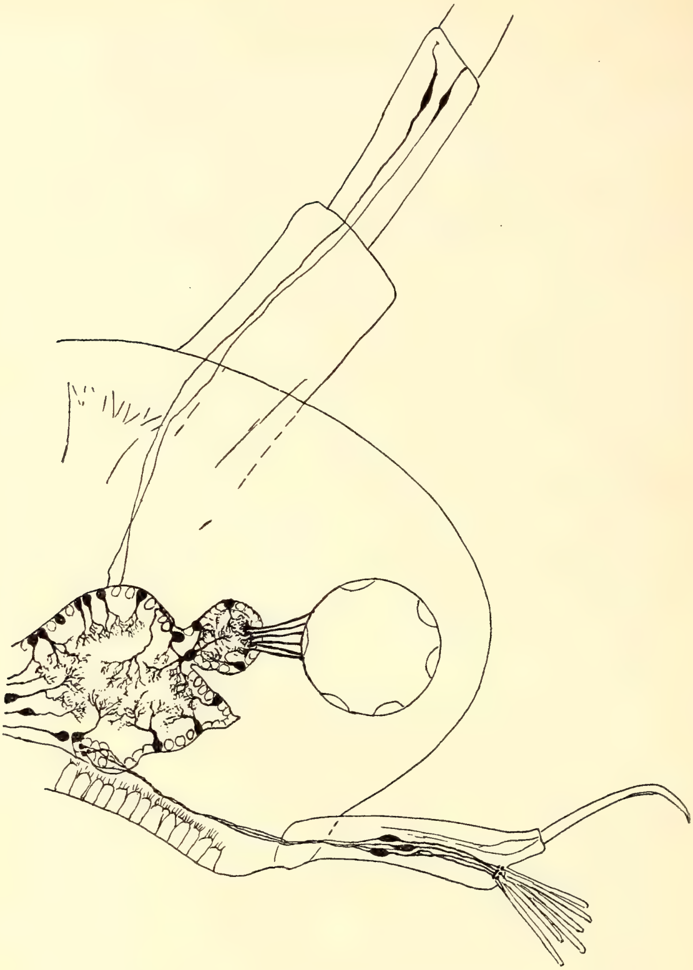
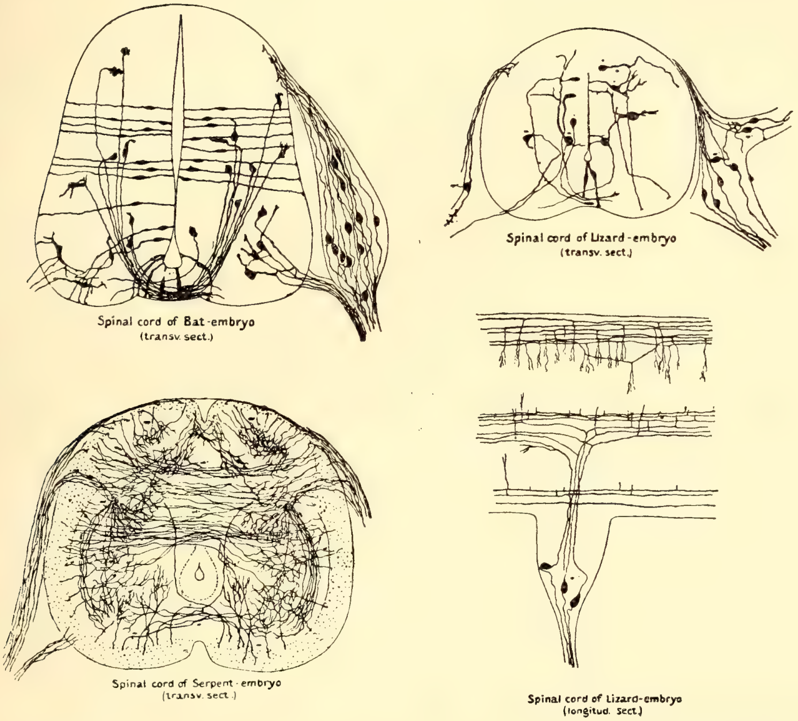


Fig. 6.—Head of small Crustacean (Daphnia, male), from the right side, showing several nerve elements coloured (black). On the left is the brain ganglion, with nerve-cells, and on the right side of it the smaller optic ganglion, in which the optic nerve-fibres, coming from the eye, end. Below, there is seen in the figure the bipolar sensory cells in the first antenna, constituting the so-called organ of smell, with central fibres going to the basis of the brain-ganglion. In the second antenna two tactile cells, with long central fibres are seen.

independent of one another, and do not enter into connection one with another. In agreement with the statement of v. Kupffer, in 1857, His observed the nerve-fibres of the embryo growing as axis-cylinder-processes of the nerve-cells; v. Kupffer and His considered, moreover, every nerve-fibre in the body, both in the nervous central organ and in the peripheral parts of the body, to be processes of that kind more or less lengthened.

This view was now also accepted by Cajal, on the basis of his own researches; by the Golgian method he showed how the axis-cylinder process grows out of the cell body in the embryo, and how at its peripheral end it has as a rule a small thickening, the so-called bud of increscence (*cono de crecimiento*), which gropes its way, as it were, to make a path for the fibre.

Shortly afterwards, v. Lenhossék, v. Kölliker, Van Gehuchten, and myself all arrived at the same opinion as a result of our own individual investigations (fig. 7). We all of us also accepted, more or less definitely, the idea propounded by His regarding the morphological independence of the nerve-cells in their first inception. Cajal led the way here, too, and the rest of us embraced the same theory on the basis of the results which we had obtained by the Golgian method. We did not discern, as Gerlach claimed, a network in the grey substance of the spinal cord and the brain by a union of the dendrites of the nerve-cells; nor did we find, as Golgi assumed, and still seems to assume, any network which has arisen from a union of the collateral branches of the axons of the nerve-cells.


Spinal cord of Bat-embryo (transv. sect.)

Spinal cord of Lizard-embryo (transv. sect.)

Spinal cord of Serpent-embryo (transv. sect.)

Spinal cord of Lizard-embryo (longitud. sect.)

Fig. 7.—Three transverse Sections and one longitudinal Section of the Spinal Cord with Nerve-cells and Nerve-fibres stained by means of the Golgi method.

As above stated, I also did *not* find by the methylene method *any network* in the grey substance (Punktsubstanz) of the invertebrata, *but only a twistwork,* a plexus, of delicate branches of nerve-cell processes, intricately intertwined.

So we were all prepared, in accord with Cajal's definite pronouncement, to regard the connection between the various nerve-cells and their processes as effected, *not by continuity but by contact.*

In his excellent and concise review of the investigations in this department during the previous years, Waldeyer, in 1891, called this conception of a nerve-cell as an independent unit a *neurone,* a term which has since been generally adopted to imply the independence of the nerve-cells.

This doctrine, *the neurone theory,* as enunciated by Waldeyer, appeared to gain more and more ground in neurology. His', Cajal's, v. Kölliker's, v. Lenhossék's, Van Gehuchten's, and my own investigations had given the basis to and had confirmed this theory in many directions.

As for the *retina,* Dogiel described the nerve-cells as anastomosing, but Cajal's investigations by the Golgian method gave, as to this point, quite different results from Dogiel's. And as regards the peripheral nerve-ends I may say that, though I have carried out extensive researches upon them, I have never been really able clearly to see any formation of network, either among the terminal ramifications of the different neurones or in those of one and the same neurone. Thus, I have never been able to observe a reticulum between the terminal ramifications of a motor nerve-fibre on a muscle, nor in those of a sensitive nerve-fibre, nor on a nerve-fibre of one of the sensory organs. Supposing such a connection were really to occur, as it is described by some histologists, I must regard a reticulum of that kind as rather abnormal, due to some sort of secondary coalescence. I have considered myself bound to specially notify these facts, as they are of importance for the neurone theory as a whole.

The question of the relation and connection of the peripheral sensory cells to the nerve-fibres is also of fundamental importance. Scientists have long assumed, and in some cases asserted that they had observed, that in the higher animals each of those cells was directly connected with a nerve-fibre. That was held to be the case with the organs of *smell* and *taste,* and also with regard to the organ of *hearing.*

Now, how was this fact to be explained by the principles of the neurone theory? This question came forward: Can a nerve-fibre be directly connected with both a central and a peripheral cell, or be actually a process of both of them?

This important question, thanks to the researches of the last two decades, found a perfectly natural explanation and one that fits in with the neurone theory. The different sensory organs are *differently* constituted, are constructed on different plans (fig. 8).

First, as regards *the organ of taste:* A. Key, who was working at the beginning of the sixties under Max Schultze, stated that he had seen in the frog a direct connection between the ends of the nerve-fibres and certain sensory cells situated on the surface of the so-called taste-papillæ; and in the higher animals, the mammals, the same mode of termination was taken almost for granted. I was enabled by the aid of the methylene method and, later, also by the Golgian method, to demonstrate conclusively, in 1888

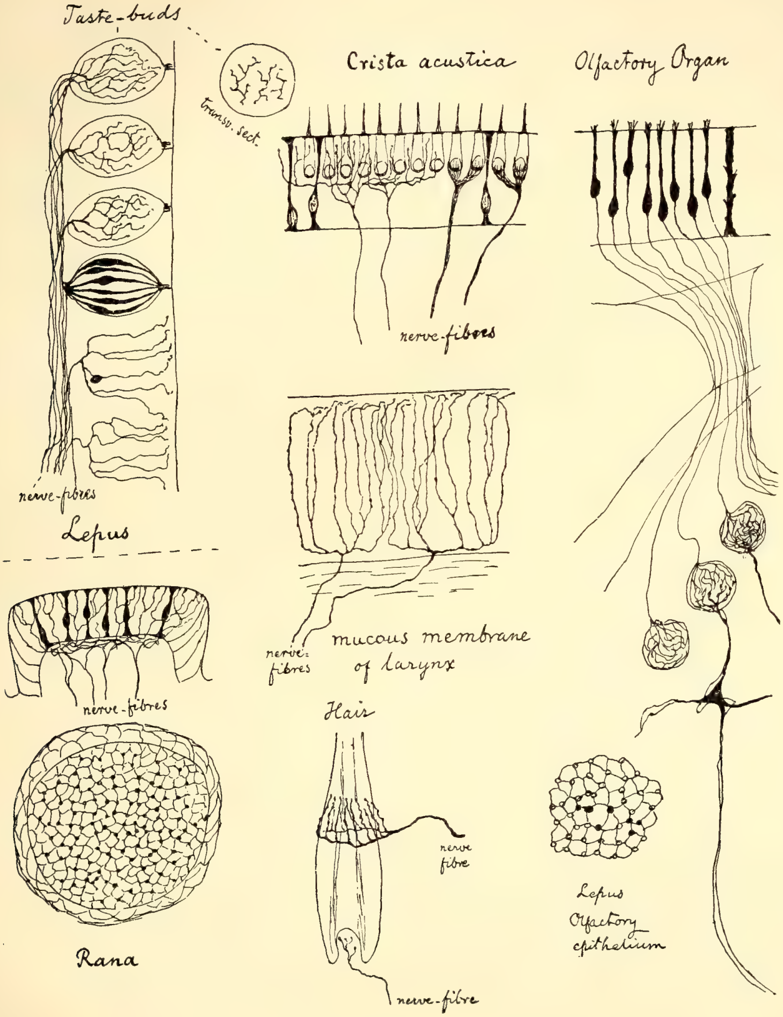

Taste-buds
transv. sect.
Crista acustica
Olfactory Organ
nerve-fibres
nerve-fibres
Lepus
nerve-fibres
mucous membrane of larynx
nerve-fibres
Hair
nerve fibre
Rana
Lepus
Olfactory epithelium
nerve-fibre

Fig. 8.—Peripheric Nerve-endings in the different Sensory Organs, stained by means of the Golgi method.

and 1892, that no mode of termination of that kind exists, that the nerve terminations find their way into the taste-buds, and copiously ramify there among the taste-cells without coming into direct connection with them.

As regards the *olfactory* organ, it could be shown clearly and distinctly by the Golgian method that the olfactory cells of the mucous membrane send out a central process which makes its way into the glomeruli of the olfactory bulbs, and there copiously ramifies among the ramifications of one or more other axons proceeding from the central nerve-cells, forming with them the glomeruli. That has been shown already by Golgi, and was confirmed by Cajal, Van Gehuchten, myself, and Kölliker; the four last named did not discover any direct connection between the nerve-fibres that proceed from the centre and those from the periphery, though that there is none is often difficult to prove for certain by reason of the exceedingly complicated convolutions of the fibres.

The sensory cells of the olfactory organ are to be regarded as a species of *peripheral nerve-cells.* They must also be regarded as corresponding fundamentally to those bipolar cells in the skin of the invertebrata already mentioned above.

In 1881 to 1884 I had shown that in the maculæ and cristæ acusticæ of *the auditory organs* the nerve-fibres enclose the lower ends of the hair cells by means of calyx-shaped extensions, and even send out fine fibrils that proceed in an upward direction on the surface of these cells. Here the matter proved more complicated, for by the Golgian method I succeeded in 1892 in proving definitely that the terminations of the auditory nerve come to an end with free ramifications among the hair cells, and, to some extent, find their way up to the surface of the epithelium. More recently Cajal has shown by his new silver method that both modes of termination, calyx formation and free ramification, exist side by side in cristæ acusticæ.

It is in any case plain that sensory cells of the auditory organ (the hair cells) may in principle be compared with those of the gustatory organ. Their development makes manifest, too, that they do not send out any fibres which proceed towards the centre, but that the bipolar nerve-cells situated in the ganglion acusticum dispatch their peripheral processes to the sensory cells in the epithelium.

By reason of these circumstances, constituting as they do an essential difference, I here distinguish sensory cells such as those of the olfactory organ, which I call *primary,* from sensory cells such as those of the gustatory and auditory organs, which I call *secondary.* The former are, of course, a species of peripheral nerve-cells, the latter are not.

I do not propose here to enter upon a discussion of the complicated structure of the organ of sight, the *retina*, which is the less requisite as Cajal described it in his Croonian lecture.

As regards the termination of the *sensitive* and tactile nerves in the epidermis, the epithelia, and the specific sense organ, the Pacinian corpuscles, the Krause end-bulbs, etc., one may formulate a general rule as follows:—They terminate everywhere with free endings and ramifications, though differing in arrangement in different cases, and sometimes among cells (Merkel's terminal discs, Grandry's corpuscles, etc.), which may, to a certain extent, be compared to secondary sensory cells.

To give a survey of these different types of terminations I have drawn up a few diagrams of the various kinds of sensory cells (fig. 9).

The *olfactory* cells and those *sensory* cells of the invertebrata that are here under discussion are consequently to be regarded as a kind of peripheral neurones, the *gustatory* and *auditory* cells on the other hand may not, strictly speaking, be so considered; the real neurones of the latter, as also of the terminal branches of the tactile nerves, are constituted by the nerve-cells situated in the cerebro-spinal ganglion system.

The neurone theory had consequently from a morphological point of view shown itself to be correct, both as regards the facts connected with the central organ and the peripheral one, and the Golgian method had yielded results agreeing in all essentials with those of the methylene staining one. The difference between our knowledge then and that we possessed, for instance, in 1880, was prodigious. Yet, in reality, it was a mere handful of investigators, dwelling far apart from one another, who had accomplished this work. In some quarters, even among eminent anatomists, physiologists, and especially neurologists, the work and results of those investigators were long regarded with a certain suspicion; now and then that suspicion found expression in a rather startling form. I might, for instance, read to you letters which I received from celebrated histologists abroad, who were otherwise favourably disposed towards me and my work, conjuring me in the most serious and moving terms not to go on experimenting with that wretched Golgian method which only resulted in art effects, impure chrome-silver precipitations within the tissues, and were misleading and dangerous for real scientific inquiry. Pretty much the same verdict was pronounced, too, from a specially authoritative source upon the methylene method as we applied it. From the point of view of the history of science it may be of a certain interest to note that our labours by no means met with encouragement and recognition in all quarters.

The new school of investigators found little difficulty in dealing with these antagonists.

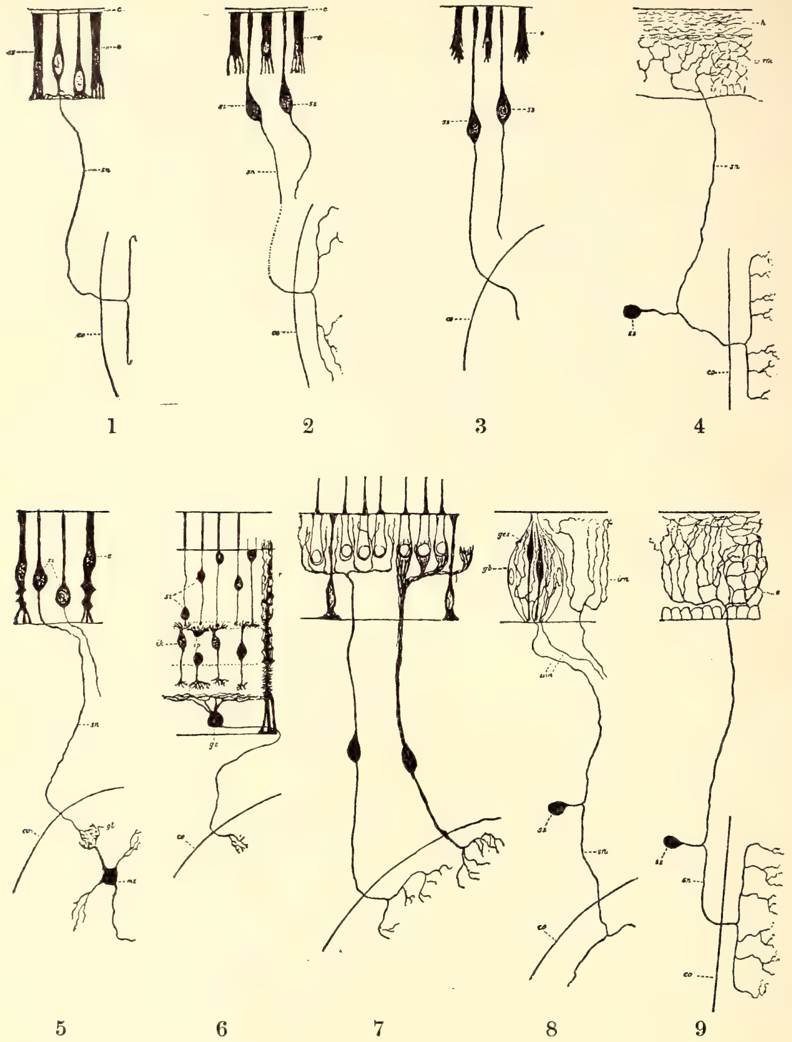


Fig. 9.—Schemes of the various Sensory Organs, with their different Sensory Cells and Nerve-endings. 1. The sensory nervous system of an oligochæte worm (Lumbricus). 2. The sensory nervous system of a polychæte worm (Nereis). 3. The sensory nervous system of a mollusc (Limax). 4. The sensory nervous system of a mammal (the skin). 5. The organ of smell in the Vertebrata. 6. The organ of sight in the Vertebrata. 7. The auditory organ in the Vertebrata. 8. The organ of taste in the Mammals. 9. The tactile organ in the Vertebrata.

But other opponents now came forward who had devised new methods and arrived at other conclusions regarding the minute structure of the nervous system. First came the Hungarian zoologist Stephan Apáthy, of Kolosvar, who after some smaller communications published a larger book illustrated by elucidating figures, entitled: "Das leitende Element des Nervensystems und seine topographischen Beziehungen zu den Zellen" (1897). By the help of a new gold-staining method he had succeeded in showing in the ganglion cells of certain worms, and especially finely in the unipolar cells of the Hirudines (fig. 10), an intracellular reticulum of fibrils which form a continuous network round the nucleus and also near the cell surface; from that network there proceeds through the single process an unbranched fibril which extends into the Punktsubstanz. In the vertebrata, too, Apáthy had found fibril network in the ganglion cells; this he described, but did not illustrate by figures.

In conjunction with this discovery of the neurofibrils, excellent in itself, Apáthy built up a whole hypothetical theory regarding the minute structure of the nervous system. Of that theory it is not easy to give a brief summary, but these are the chief points in it:—The neurofibrils discovered by him form the specific constituent of the nervous system, the nervous portion proper, the *conducting* element; they are independent structural parts; in the nerve-fibres they preserve their individuality; in three places, however, they form reticula, viz., in the ganglion cells, in the terminal organ (the sensory cells, etc.), and in Leydig's Punktsubstanz, in which they subdivide into an exceedingly intricate reticulum, Apáthy's "Elementargitter," in which the sensory nerve-fibres in particular dissolve and lose their individuality.

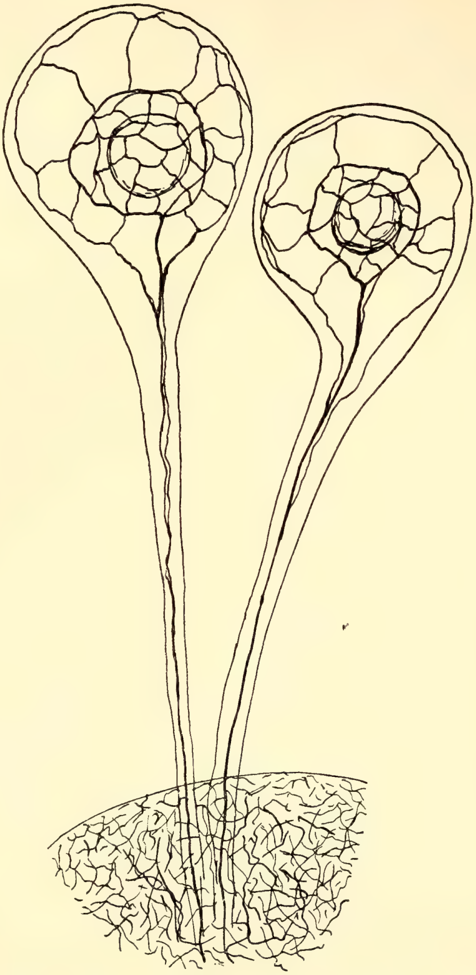


Fig. 10.—Two Unipolar Nerve-cells from a Ganglion of the Ventral Nervous Chain of Hirudo. In the cells is seen the fibrillar network of Apáthy, running down through the cell process into the Punktsubstanz, which consists of fine twisted fibres.

In connection with this conception Apáthy constructed his hypothetic theory regarding the minute structure of the nervous system. He maintains that there are two kinds of nervous cells, nerve-cells and ganglion cells. The nerve-cells produce the conducting substance, the neurofibrils, which grow both towards the centre into the ganglion cells and towards the periphery into the sensory cells, the muscle cells, etc. The neurofibrils constitute consequently an element foreign to the ganglion cells which has grown into them from without; they are further capable of independently emerging from the ganglion cells and their processes, the nerve-fibres; they do not accordingly need to remain in them and their paths. They form, moreover, large continuous reticula in the organs of the body and above all in the central grey substance. It is not to be wondered at if the specialists were somewhat astonished at this new system of Apáthy's, which appeared to them to rest on very uncertain foundations. Apáthy's own preparations of Hirudo ganglions were exhibited at zoological and anatomical congresses. The existence of the intracellular fibril network in the ganglion cells of certain worms was clearly apparent in these preparations, but his "Elementargitter" in the Punktsubstanz, on the other hand, was not confirmed; there did not appear a reticulum, but a plexus of non-anastomosing delicate fibres.

However, Apáthy was now to obtain an ardent supporter in a German physiologist, A. Bethe, who declared himself a believer in his theories. This scientist, who had been experimenting especially with the methylene staining method and had invented a good fixative for the preparations resulting from it, devoted himself to the question of the occurrence of fibrils in the ganglion cells of the higher animals, a question which Max Schultze had previously done a good deal to elucidate. Bethe invented a new method of staining these fibrils, which he represents as non-anastomosing among themselves as a rule. Bethe very vigorously championed the main article in Apáthy's theory, both in lectures and essays, and also in a large book on the subject; he was a particularly strong upholder of the idea that the nerve-fibrils are the true conducting element in the nerve-tissue and of their independence. Bethe became one of the chief opponents of the neurone theory and, generally speaking, of the school of ideas of which Cajal was the leading force. Bethe exercised for a time a not inconsiderable influence upon many of the Neurologists' party. Some of the results he obtained by his experiments were especially effective in strengthening his position as a supporter of Apáthy's theory. Bethe had removed certain groups of ganglion cells from some live crabs (*Carcinus mœnas*), but could nevertheless observe reflex activity in the processes of the nerve-cells belonging to them. Thus, he alleged he had hereby clearly shown the small importance the ganglion cells have as regards the activity of the nerves.

I have myself tried to carry out these experiments of Bethe's on my own account, but have come to the conclusion that it is practically impossible to operate with such precision on the semi-transparent and soft ganglions of the living animal (*Carcinus mœnas*) that a removal of some particular groups of cells will be certain to result. Under such conditions I consequently regard it as exceedingly unsafe to draw the conclusions Bethe has done. The question is fundamentally of such moment that the utmost caution is necessary in arriving at any conclusions at all. I, at any rate, after the experience I have derived from my experiments, must call in question the certainty and accuracy of what he has stated as his conclusions.

Now, however, a new epoch of neurological investigation was to be inaugurated, for Cajal and Bielschowsky almost simultaneously invented methods very similar to one another for staining the fibrils in the nerve-cells and their processes by means of a silver solution. That not only confirmed the existence of the neurofibrils seen by Apáthy, but added very materially to our knowledge, especially of the nervous system of the higher animals, the vertebrata. Thanks to these methods, it was shown by the inventors themselves and by other investigators, who had taken up the study of these problems, that everywhere in the nerve-cells and their processes there exist fibrils which belong to the cell substance and are very early developed in it. Cajal proved, too, that these fibrils form reticula in the cells, and that they increase or decrease in thickness according to the state in which the animal is, different in health and sickness (*e.g.*, rabies), etc.; he also pointed out that in the cell processes these fibrils always remain within their substance, and are not, as Apáthy asserts, capable of emerging from it. Cajal asserted also that the fibrils do not anastomose outside the neurones, and specially not in the central substance and in the Punktsubstanz. This important point, which entirely agrees with the results I obtained before by the methylene method, was confirmed by the new researches I carried out with Cajal's new fibril staining method which gives a very full and distinct colouring to even the most delicate ramifications of the nerve-fibres (fig. 11). Now these form everywhere, even in the most successfully stained parts, in the Punktsubstanz of the invertebrata as well as in the grey substance of the vertebrata, *not* anastomosing *reticula, but only twistwork* (*plexus*); divisions in the fibres occur, but *no network* is visible.

I call attention to this fact once more, since the question has an important

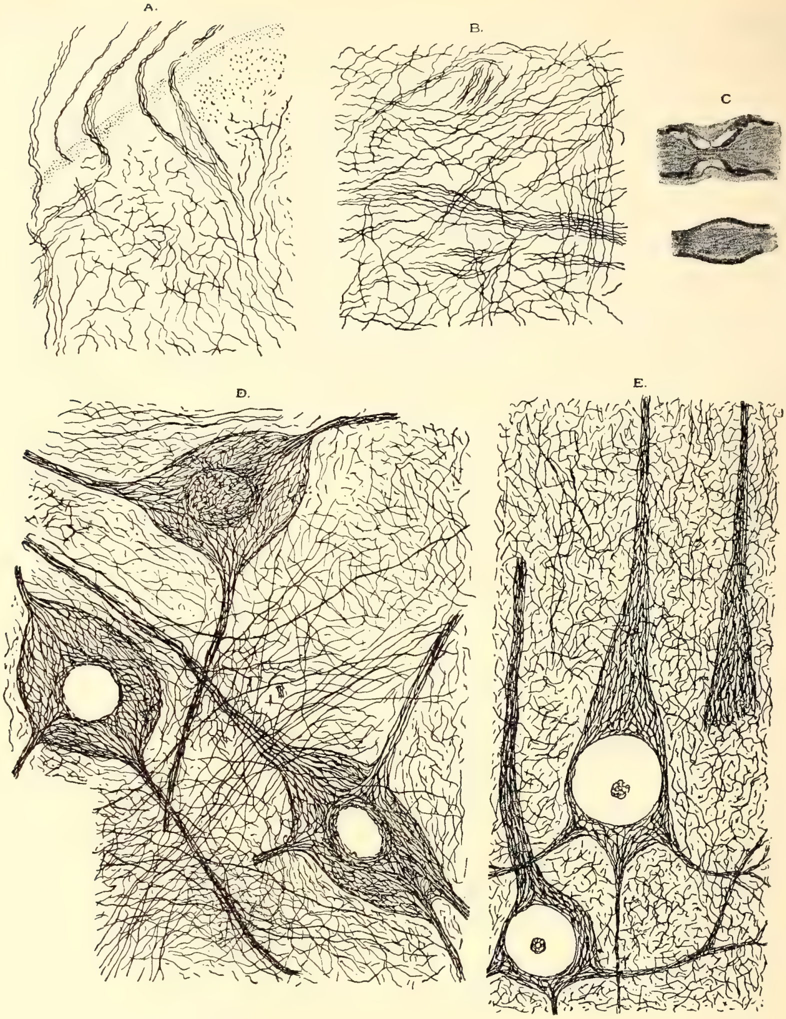


Fig. 11.—The Intracellular Network and the Intercellular Twistwork in the Grey Substance of the Central Nervous Organs. A and B, the Punktsubstanz in the ganglions of Hirudo (twistwork); D and E, nerve-cells in the spinal cord and the cortex of the brain of a rabbit. In the cells is seen the intracellular network of fibrils, and between the cells is shown the twistwork of intercellular fibrils.

bearing on the neurone theory and on our ideas regarding the construction and function of the nervous system as a whole. Those groups of scientists who have proclaimed the presence of reticulum cannot in this particular find support in the results even of their own methods. I have myself seen thousands of excellent preparations produced by the Golgian method, but have never seen real reticula in the grey substance of either the higher or the lower animals. I have carefully studied a series of preparations produced by both Apáthy's and Cajal's methods—even the preparations made by Apáthy himself—but have never succeeded in discovering the reticula which they maintain as being present.

Apáthy, however, and his followers state that this is simply due to the staining not having been perfect. In that case all the stainings that have been submitted to our examination have been imperfect, *including even Apáthy's own.*

A scientific investigator is in duty bound to confine himself to describing what he really sees; he has no right to formulate *theories* on the basis of what he does not see, but only imagines and assumes that he may see. It is, of course, true that our power of sight is limited, and that even within the range of our vision there may be much of which we have no knowledge. We may form hypotheses and conceive probabilities concerning those regions which are invisible to us, but we must be very careful not to confuse those fancies with the exact results of science. Consequently, it will not be possible for scientific investigators to accept the existence of anastomosing reticula in the grey substance as a fact, and to build up scientific theories on that basis until reliable methods have proved it to be so.

The sum of what has hitherto been shown by the investigations in this field is, according to my opinion, this only: That neurone fibrils are to be found in the nerve-cells and their processes, that they form *in the cells* abundant reticula, which are even plainly to be seen in some of the peripheral terminal organs, and that they do not anastomose *outside* the particular domains of the cell unit or neurones, *i.e.* that they *do not outside these form reticula but plexus.* The several neurones are connected one with another *per contiguitatem*, not *per continuitatem.** Finally, there does not exist any certain proof that the fibrils constitute the sole conducting element.

I know very well that there are instances described where there are observed anastomosing of nerve-cells, but these cases are really very few, and may be, if they are certain, considered as abnormal, and, at all events, very rare. The question as to the independence of the neurones one of another, or their continuous connection one with another by means of fibrilliferous anastomoses, either from cell body to cell body or from process to process, has once more come to the front during the last few years and divided investigators into two different camps. As above mentioned, it was shown by His that the nerve-cells of the central organ do not anastomose with each other during the first stage of their development, but are independent cell entities. This has been confirmed by most of the specialists (Cajal, v. Kölliker, v. Lenhossék, myself, Van Gehuchten, etc.), who have made an intimate study of the development of these organs in the embryo.*

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* During the preparation of this lecture for print I have received from the author, Dr. Em. Mencl, in Prag, a memoir on the "Punktsubstanz of the Hirudinees." His researches have led him to the same conclusions about its structure. "Ich habe," he says at the end of the memoir, "das Verhalten der Neurofibrillen und der Zellausläufer wiederholt einer eingehenden Untersuchung unterworfen, natürlich, wie erwähnt, an nach Apáthy und Cajal behandeltem Material, hauptsächlich aber an den Cajal'schen Präparaten, wo die Reaction vollkommen gelungen war—und trotzdem habe ich nie irgendwelche Netze, ausser den intracellulären Körbchen, gesehen, weder in den Verlauf der Ausläufer eingeschaltet, noch in der centralen Masse. In dieser Hinsicht kann ich direkt mit Retzius sagen: 'Auch bei den stärksten anwendbaren Vergrösserungen sah ich in der Punktsubstanz nie ein Netz, nur ein Geflecht, keine Anastomosen der Äste.'"

There has long existed a theory diametrically opposed to that of His, respecting the origin of the peripheral nerve-fibres; it was first brought forward by the great embryologist Balfour, and has been since vigorously upheld by a number of other investigators. Their theory is that the nerve-fibres owe their formation to special cell chains which are to be found in the various parts of the body from the beginning, or, at any rate, very early.

Among the supporters of this cell-chain theory, however, one, the renowned embryologist Dohrn, has lately announced, that on the strength of his most recent investigations, he has been obliged to give up his earlier opinion entirely, though he had been convinced before of its correctness. Dohrn must therefore not be counted any longer as an exponent of the chain theory. Oscar Schultze is at present the foremost upholder of this theory. The results of several investigators, however, are at direct variance with his. The last researches of v. Kölliker, completed just before and published shortly after the death of the great anatomist, prove with convincing clearness that the nerve-fibres grow out of the central organ towards the periphery. Cajal's numerous researches tell the same story.

But still more conclusive, if possible, is the work of R. G. Harrison, the

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* Among the adherents of the neurone theory, the Russian neurohistologist Dogiel, who, especially with the methylene blue method, has made brilliant discoveries in the field of the peripheral nervous system, has pronounced (1904) a theory which is somewhat different from that of the other neuronists. Dogiel thinks that in the central organs certain colonies of nerve-cells form units by means of their dendrite processes: here "verbinden sich somit die Nervenzellen einer Art vermittelst ihrer Dendriten zu Zellkolonien; die in den Bestand aller Zellen einer Kolonie eingehenden Neurofibrillen bilden Reihen von geschlossenen und eng miteinander verbundenen Netzen, welche mit dem Neurofibrillensystem anderer Kolonien, mit welcher die erstere funktionell verbunden ist, organisch nicht zusammenhängen."

In the central organs of the higher animals, I do not know of any proved instances of such colonies of nerve-cells, and in the lower animals I have never found them. This year Dr. Deineka, working in the laboratory of Professor Dogiel, in his researches on the nervous system of Ascaris, has come to the conclusion that the motor and sensory systems do not unite with each other *per continuitatem*, but by contact; the sensory cells, however form organic continuities with each other.

American histologist, who has shown by a series of clever and successful experiments on frog larvæ that the nerve-fibres grow out of the central organ. Harrison was, in fact, able to trace this process under the microscope in the living tissue and could even determine how much the fibres grow in a minute (about half a micron in the minute). By experimental methods he was actually able to check or delay the migration from the central organ of these cells which form the so-called Schwann's sheaths round the peripheral nerve-fibres. It is these Schwann's sheath-cells which are regarded by supporters of the chain-theory as the mother cells of the nerve-fibres ; but Harrison was able to show that nerve-fibres will grow even in cases where no sheath-cells exist. Oscar Schultze has tried in his last treatise to minimise the value of these results of Harrison's, which we others consider almost conclusive, by stating that they are due to the attack of the experimental method, and do not therefore represent normal conditions. If that line of argument is to be allowed, then the results obtained by experimental interference with natural processes in general must be rejected, which would mean that a considerable part of the science of physiology would have to be rejected as unreliable, as being essentially based upon experimental interference of that kind. It is in this direction that so many excellent scientific discoveries have recently been made and upon which are centred the greatest hopes of progress for the future.

To this group of phenomena and the results yielded by them we must assign the correct conception, arrived at by study, regarding *the regeneration of the nerves* after having been traumatically injured. It would carry me too far, if I were to attempt to give a detailed account of the numerous and extensive experimental researches upon this highly remarkable and involved process. After having been, with great success, made the object of research for a long time past by a group of distinguished physiologists, Waller, Vulpian, Langley, Sherrington, Vanlair, to only mention some of the more prominent ones, the question was also taken up by the modern histologists some years ago and has since been investigated by their methods ; Cajal and Perroncito, experimenting quite independently of each other, arrived at very nearly coincident results.

It has long been known that, if a nerve be severed, the central and the peripheral portions will reunite and the nerve be restored to use ; the same regeneration will take place even if a piece be cut out. It had been generally assumed after the date of Waller's renowned investigations that nerve-fibres which are wholly or in large measure separated from the cell body with which they have been in direct communication, will degenerate if they do not speedily regain their connection with it. The German physiologist Bethe, however, maintained some years ago that he had succeeded in showing, by experiments upon living animals, that a peripheral nerve portion, when separated from its cells, is capable of regenerating of its own accord, the sheath-cells of the fibres being what really effect the regeneration.

This discovery aroused a great deal of attention, and was regarded in many quarters as dealing a decisive blow at the neurone theory. The researches, which several other scientists (Braus, Van Gehuchten, etc.) carried out, seemed to confirm Bethe's results. On the other hand, fresh researches made by Cajal and Perroncito, most carefully and accurately, soon proved that Bethe had not exercised due care in the researches he undertook, and that the conclusions which he drew were hasty and incorrect. It was plainly shown by Cajal's and Perroncito's numerous experiments that regeneration proceeds solely from the central nerve-portion, the fibres of which still remain in direct connection with its central nerve-cells, and that the nerve-fibres of the peripheral nerve-portion degenerate; that these however, after a time, are replaced and regenerated by the fibres of the central nerve-portion, which grow towards the peripheral nerve-portion, and replace its degenerated fibres (fig. 12). A regeneration of this kind, issuing from the central nerve-portion, may be effected at surprisingly long distances, and in spite of great obstacles, even through the length of muscles that lie in the way. It is as if a chemotactic law governed the outward growth from the central nerve-portion to the periphery, as indeed was before shown by the experiments of the Swedish pathologist Forssman, in Lund, and still earlier by the experiments conducted by the French histologist Ranvier, upon the nerves of Cornea, which quickly regenerated after destruction, so that Ranvier considered himself justified in establishing a law of the growth of the nerves towards the periphery.

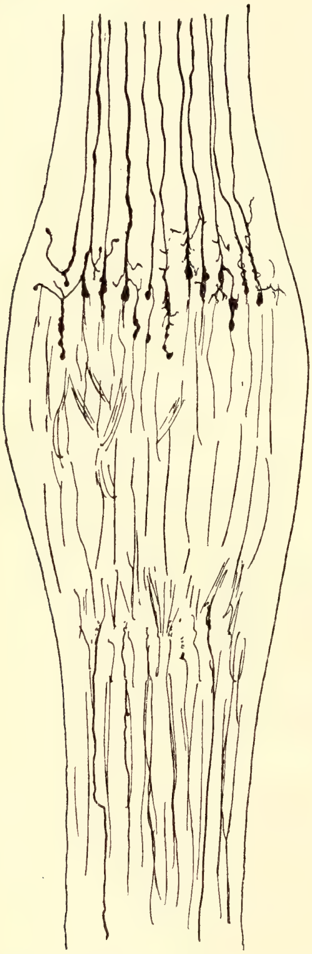

Fig. 12.—Scheme of the Regeneration of a Peripheric Nerve. The upper part is the central portion, the lower part is the peripheric portion; in the midst is the damaged portion.

Owing to results thus obtained by experiments with nerve regeneration, the just mentioned objections raised to the neurone theory as a result of the more or less dubious experiments of Bethe and others, and their incorrect interpretation, which for some time were listened to by the scientific world, have thus been refuted.

As far as I can see, the neurone theory, in its main features, has issued victorious from the strife between its supporters and opponents. One may still acknowledge that the latter have rendered good negative service, on the whole, by their opposition, for they have continually prompted others to new researches which have led to the problem being more and more thoroughly cleared up.

The pictures to be obtained by a study of regeneration, as evinced by the central nerve-portion, are, besides, very remarkable. The severed axons send forth finer or coarser branches, which grope their way onwards along the strangest paths and in varying forms. It may be said, in a certain sense, that the description by Cajal and Perroncito forcibly reminds us of the purely normal growth in the embryo of the axons of the central nerve-cells and their buds of increscence, etc. But they also remind us of the pictures presented by a large number of the so-called sensitive terminal organs in their normal occurrence in the adult individual. The Swedish anatomist Ramström, of Upsala, who has been subjecting these organs, Meissner's and Ruffini's, and the Pacinian corpuscles, Krause's end-bulbs, etc., to a close and critical scrutiny, in order to establish their significance, both morphological and physiological, has already arrived at very remarkable results, which may throw light upon the real nature of these bodies and upon their origin and functions.

There remains, however, to be mentioned another opponent to the His-Cajal-Waldeyer neurone theory, in its original form: Held, the German histologist, now the real leader of its antagonists. He had tried previously to show the occurrence of fine terminal ramifications and end-bud formations, by which the ends of some nerves embrace certain nerve-cells, and are described as being in direct connection with their substance.

By a study of the first occurrence of the inner fibril reticulum of the nerve-cells, Held formed an opinion as to the first formation of nerve-fibres in the embryo, which essentially resembles the one pronounced by the German physiologist Hensen, many years ago. Hensen's doctrine was that the nerve-fibres do not grow towards the periphery, but are connected from the very beginning both with their central nerve-cells and with the terminal organ in the periphery.

Held has now conceived the idea that the only thing that grows towards the periphery is the intracellulur fibril network itself. He has shown that it is formed very early in a special part of the nerve-cells (neuroblasts), subsequently growing towards the periphery (and towards the centre) along the previously formed paths. He maintains also that there are a large number of anastomoses between the special nerve-cells, and even between their processes; these he calls *neurodesms,* and says that the fibrils in them are able to grow from one neurone to another; in that way a large anastomosing fibril network might arise in the organism, somewhat similar to the one Apáthy has proclaimed.

In a criticism of this theory of Held's, which Cajal published recently, he showed forcibly its inadmissibility. For my own part I must join Cajal in his estimate of this attempt of Held's to alter the neurone theory. However, I have no opportunity now of entering into a further discussion of the matter, and so must only refer to Cajal's critique.

In addition to the researches, the progress and present results of which I have here endeavoured to give a brief sketch, there are a few other branches of this extensive field of work that are of great importance for the science of Neurology, which, however, the time at my disposal does not permit me more than briefly touching upon.

One of these lines of investigation is directed towards finding out accurately the finer structure of the cortex substance and the nuclei of the inner ganglia of the brain, with special attention to the exact discovery of the characteristic structure of each several part of it. In this inquiry the aim has been first and foremost to find the distinguishing marks of the different centres in the brain cortex, in accordance with what Physiology teaches us. There came first some few scattered observations of a tentative nature, then, above all, Meynert's subdivision of the brain cortex into five strata on the basis of the differing constitution of the nerve-cells, Betz's discovery of the large pyramid cells, and Bevan Lewis's analysis of their occurrence, which also embraces investigations of Gyrus hippocampi and its special construction. In 1893 the Swedish neurologist Carl Hammarberg, whose early death was a real loss to science, published the results of his systematically conducted studies upon the cortex substance of the human brain, and thereby paved the way for a more exact special investigation into this important department. Golgi, Kaes, and others carried the inquiry still further, but it was reserved for Cajal to effect a real enrichment of our knowledge in this direction as a result of his subtle and accurate researches. The work done by Cajal has also brought to light many important points regarding structure in respect to the ganglia of the brain, as has that of v. Kölliker, of Van Gehuchten, and of several other scientists, but in those departments there still remains a great deal to be done before the anatomical structure of the parts in question can yield a sure basis for a true conception of their several functions. In the first place, the investigations have been concerned with discovering the special structure of the sensory centres physiologically best known, viz., the optic, olfactory, and auditory centres, and here remarkable discoveries have been made.

The other important line of investigation has been directed towards finding out the course taken by the bundles of nerve-fibres through the substance of the brain and the spinal cord. This problem, so exceedingly important, indeed fundamental, for Physiology and Pathology, has long been one of those which anatomists were most of all desirous of solving, first by macroscopical dissection of brain substance specially hardened for the purpose, and then by an examination of coloured serial sections. In this work the colouring method invented by Weigert has played an important part, as has that of Marchi, but by no means less momentous have been the researches, so ingeniously and energetically pursued, first of all, by Flechsig, into the myelinisation (*i.e.*, the formation of the myelin sheaths) of the different bundles of nerve-fibres which takes place at varying times. The exceedingly important results at which Flechsig arrived on the basis of these investigations have led up, as far as the construction of the cerebral cortex is concerned, to his well-known subdivision into different association-centres.

Time would not allow of my endeavouring to give even a brief survey of these association-centres or of the nerve-fibre paths that lead to and from them; I must also pass by, in silence, numbers of researches carried out by various investigators, some by anatomical, some by experimental, methods, and others, again, by a study of cases of disease, though they have yielded a large quantity of excellent and, to some extent, brilliant results.

Among other fields of inquiry in the large domain of modern neurology there are deserving of mention here: the remarkable transplantation experiments made by Braus, Harrison, Nageotte, and others, which are evidently in affinity with the nerve generation experiments. Furthermore, there should be mentioned the researches that have been instituted by Edinger and his school of investigators into the phylogenetic development of the construction of the brain in the lower vertebrata from Amphioxus upwards in the chain of animals, researches which have already yielded several excellent results and which promise to illuminate many of the hitherto dark points in reference to the organisation of the central nervous system.

However much may be said to have been accomplished by the assiduous investigations pursued during recent decades to elucidate the minute structure and composition of the central nervous system, there remains, nevertheless, an almost infinite amount to be done in this domain. Almost every year that passes witnesses the rise of new opinions and the discovery of new and unsuspected details of structure. For instance, who would have been able to foretell some years ago that in the spinal ganglions, which to all appearance are so comparatively simple in their structure, such complicated ramifications and connections would be found to exist in the ganglion as those which have recently been made manifest by Cajal and by Dogiel? There still remain without any doubt innumerable discoveries for coming generations in all those domains.

The limited time for this lecture does not permit me to enter any further upon these fields.

When it is remembered, however, what difficulties present themselves to the investigator, it is surely a matter for astonishment that our knowledge has been advanced in so splendid a way during the recent decades, more especially in the last two. This fact warrants us in cherishing the confident hope, that by means of sedulously pursued, thoroughgoing labour on the part of scientists in all civilised countries, the secrets of the nervous system may be brought nearer to light. Still we must not imagine that the truths which we believe to be established are absolutely and unalterably certain. In science, the famous words of Duclaux, the French scientist, will always remain true:

> "La science s'avance parce qu'elle n'est sûr de rien."

These words have a wide and profound significance. New methods, new researches, often teach us to see things in a new and to some extent different light, and may even disclose errors in results which have been long accepted and approved.

I am not myself willing to go so far as Carl Ludwig, the renowned German physiologist, when he said: "Die Methode ist Alles," though I admit that there is a good deal of truth in his words. New and good methods carry our knowledge forward, but moreover the investigator requires that perseverance, that devotion and that critical acumen in observation, which, provided he is not a prey to preconceived ideas, make it possible for him to distinguish between appearances and reality. Hypotheses may be excellent as lodestars though they may on occasion lead one astray. Theories can only hold their ground and lay claim to consideration as long as they agree with the facts which have been brought to light and constitute a comprehensive summary of their results; if that is no longer the case they must inevitably fall and yield place to others, in the light of the criticism brought to bear upon them and in obedience to the new observations which science has made.

It seems that these theses do contain such general truths that all may agree about them. But the history of our science shows that there are many digressions from them. We must therefore all be prepared to give up our theories when these are shown to be incorrect. With regard to the *neurone* theory, I also am willing to do this as soon as it is really proved that it does not agree with the certified facts. But that is certainly not yet the case.

The researches of which I have had the honour to give an account to this illustrious audience have in view the study of the construction of the organ of psychic activity, *the nervous system* and above all *the brain.* The goal they seek to arrive at is one of the utmost difficulty, if not, indeed, in the whole of its compass, impossible of attainment. Science cannot stand still. Ultimately the object of the investigation is *ourselves,* the *human brain,* the organ of our own mental being.

Here, above all, the words of the great English poet, Pope, are applicable—words subsequently reiterated by Göthe:

> "The proper study of mankind is man."

A Study of the Variations in the Secretion of Hydrochloric Acid in the Gastric Contents of Mice and Rats as compared with the Human Subject, in Cancer.[*]

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In March, 1905, Dr. Benjamin Moore, in collaboration with Messrs. Alexander, Kelly, and Roaf, communicated a paper[†] to this Society on the "Absence or Marked Diminution of Free Hydrochloric Acid in the Gastric Contents in Malignant Disease of Organs other than the Stomach." The conclusions arrived at were based on the analyses of test meals given to human subjects.

Later in 1905, Dr. Willcox, of St. Mary's Hospital, published a paper on the same subject in the 'Lancet,'[‡] and his results appeared to confirm generally those of Dr. Moore. Other workers, among whom Dr. Morton Palmer[§] may specially be mentioned, followed on the same lines, and the matter attracted considerable attention among physiologists.

It occurred to us that in connection with the extensive researches in progress at the laboratories of the Imperial Cancer Research Fund, there was a mass of material ready to hand in the large number of mice under observation there. Accordingly we approached the Director of the Laboratories, Dr. Bashford, on the subject, with the suggestion that we should endeavour to control observations on the human subject by determining the physiologically active hydrochloric acid both in the stomachs of normal mice and in those of mice the subjects of transplanted tumour growth. Dr. Bashford most kindly acquiesced and offered to place any necessary materials at our entire disposal.

The only chemical work that had been done in connection with mice was by Dr. Cramer,[||] in the laboratory of the Imperial Cancer Research Fund, who, in 1905, made some estimations of the total acidity in the stomach contents of mice inoculated with alveolar carcinoma (Jensen), and had found that there was, apparently, no decrease in this acidity as compared with normal mice.

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[*] Towards the expenses of this research a grant was received from the Executive Council of the Imperial Cancer Research Fund.

[†] 'Roy. Soc. Proc.,' B, vol. 76, 1905, p. 138.

[‡] 'Lancet,' June 10, 1905, p. 1566.

[§] Guy's Hospital Reports, vol. 60, 1906, p. 181.

[||] 'Biochemisches Centralblatt,' vol. 4, p. 65, June, 1905.

We commenced our experiments in December, 1905, and have continued them with comparatively little interruption up to the present time. At the beginning of this year (1908) we were fortunate in obtaining a grant from the Imperial Cancer Research Fund, which, by enabling us to retain the services of a skilled assistant, rendered possible the completion of our work, in the course of which, during the last two and a-half years, we have had the opportunity of examining over 1000 stomachs of normal and of "cancer" mice.

Our primary object was to ascertain whether the suggested absence or marked diminution of free hydrochloric acid in the stomachs of human beings suffering from cancer occurred also in mice suffering from the same disease, whether spontaneously arising or the result of experimental transplantation. Obviously the *method* to be adopted for the determination of the hydrochloric acid was an important consideration, and after careful study of the various processes used by other investigators in similar work, we finally determined to employ Volhard's method for the volumetric estimation of chlorides, the principle of which is based on the precipitation of the chloride by an excess of standard silver nitrate in the presence of free nitric acid and the subsequent determination of the excess of silver nitrate by means of ammonium thiocyanate, using iron alum as an indicator.

This method was ingeniously adapted by Lüttke,* and further simplified by Willcox, for the purpose of estimating hydrochloric acid in gastric contents, and we have followed these adaptations with such further modifications as seemed to us necessary for our particular purposes.

The special advantage of Volhard's process, apart from its accuracy, is that the hydrochloric acid, free or combined, is precipitated as chloride of silver in presence of free nitric acid. There can therefore be no question as to the particular acid estimated, a point which has been raised, although in our opinion needlessly, in connection with the Mörner Sjöqvist method of estimating free and combined hydrochloric acid (see later).

Briefly, the *modus operandi* of the method as employed by us has been as follows:—The stomachs are counted, weighed, crushed, and macerated in water for about 12 hours, and the mixture made up to a known volume and filtered. The examination of the filtrate is then carried out in the following manner:—

(*a*) The *total chlorides* (free, inorganically and organically combined hydrochloric acid) are estimated by Volhard's method in an aliquot part.

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* 'Deutsch. Med. Woch.,' 1891, p. 1325.

(*b*) The *inorganic chlorides* are similarly estimated in another aliquot part after evaporation to dryness on a water-bath and charring the residue at a gentle heat.

(*c*) The *total acidity* is determined in another aliquot part by titration with standard sodium hydrate, using phenolphthaleïn as an indicator.

From these three determinations may be deduced:—

1. Physiologically active hydrochloric acid = $(a-b)$.
2. Organic acids = $(c-(a-b))$.

All of these various constituents are, for convenience, expressed in terms of hydrochloric acid.*

The term *physiologically active hydrochloric acid* is intended to include both the free acid and that combined with proteids and nitrogenous organic bases, since, as originally pointed out by Lüttke in 1891,† and more recently emphasised by Willcox, much misconception has arisen in the use of the terms "free" and "combined" as applied to hydrochloric acid in the gastric contents, and it has been erroneously held that the presence of the free acid is of more importance than the organically combined acid. But inasmuch as the latter may have been free a short time before its estimation, the exclusive determination of "free" hydrochloric acid cannot but lead to fallacious conclusions.

With this introductory explanation, we may now consider the summary of the results which we have obtained.

(1) *Experiments on Mice made without Reference to Time of Digestion* (*Series* 1 *to* 5, *Mice over* 12 *months old*).

The first five series of experiments (December 19, 1905, to May 7, 1906) were made without reference to time of digestion, not because we had not fully considered the possible importance of this point, but more with a view of noting, as a preliminary step, whether under conditions of ordinary feeding any appreciable difference could be demonstrated in the amount of physiologically active hydrochloric acid present in the stomachs of normal mice as compared with that present in the stomachs of mice with transplanted tumours.

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* In the estimations of total and inorganic chlorides in mice stomachs, 20 c.c. to 50 c.c. of aqueous extract were taken, according as the extract had been made up to 100 c.c. or 250 c.c. It might appear probable that a source of error would arise in the charring of the residue, due to partial volatilisation of inorganic chlorides; we tested this point rigidly by repeating the estimations in a very large number of instances, but our experience was that there was practically no error, the difference in the titration numbers being no greater than that usually found in closely agreeing estimations. The presence of the charred matter does not interfere in the end-reaction when the quantity of chlorides present is comparatively large, but is liable to cloak the reaction when the amount is small. To overcome this difficulty we found it was only necessary to add a somewhat larger excess of standard silver nitrate solution, so that when titrating back with ammonium thiocyanate the obscuring effect of the charred matter was annulled by the extra amount of white silver thiocyanate in suspension. By adopting these modifications and by using N/50 or even N/100 instead of N/10 standard solutions, we were able to determine the chlorides in a single stomach with accuracy and comparative ease.

† *Loc. cit.*

The results obtained indicated, somewhat to our surprise, an increase rather than a decrease of hydrochloric acid in stomachs of mice with transplanted tumours, as the following summary (Table A) shows:—

Table A.*—Percentage of Physiologically Active Hydrochloric Acid in the Gastric Contents of Normal Mice, and of Mice with Transplanted Tumours, without Reference to Time of Digestion.

Series.	Date.	Normal mice.		Mice with non-ulcerated tumours.		Mice with ulcerated tumours.	
		No. of stomachs.	Hydrochloric acid.	No. of stomachs.	Hydrochloric acid.	No. of stomachs.	Hydrochloric acid.
1	Dec. 19, 1905	6	0·0382	4	0·2169	2	0·2321
2	Jan. 4, 1906	19	0·1841	17	0·1003	2	0·1494
3	Jan. 31, 1906	40	0·1127	27	0·1522	10	0·1711
4	Mar. 12, 1906	45	0·1081	37	0·1133	14	0·1085
5	May 7, 1906	40	0·0930	61	0·2549	4	0·1740
Average		150	0·1121	146	0·1815	32	0·1465

Owing to the small weight of the individual stomachs (averaging about 0·75 gramme), we did not at first see our way to determining the chlorides in a single stomach, though later on (see footnote, p. 446) we were able to accomplish this with accuracy. We therefore took batches of stomachs varying in number from 6 to 60 with a view to arriving at a general comparative average. From Table A it will be seen that in the normal mice the physiologically active hydrochloric acid varied from 0·0382 to 0·1841 per cent. with an average of 0·1121 per cent.; in the mice with non-ulcerated tumours it varied from 0·1003 to 0·2549 per cent. with an average of 0·1815 per cent. and in the mice with ulcerated tumours it varied between 0·1085 and 0·2321 per cent., with an average of 0·1465 per cent.

Thus under exactly similar conditions but without reference to time of digestion, the mice being simply taken from their cages, in which there was always food, and killed at once, the mice with transplanted tumours showed an average greater proportion of physiologically active hydrochloric acid in the gastric contents, with a less variation between the extremes, than was the case with the normal mice.

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* The tumours in these series were all alveolar carcinoma (Jensen), and varied in weight from 0·2 to 10 grammes.

It is only right to state that in the case of the mice with tumours referred to in Table A, although they were fed and killed as above stated, the stomachs were not sent to us at once, but kept, in an ice safe in glass tubes with cotton wool stoppers, for periods varying from a few days to a few weeks; the stomachs of the normal mice, however, killed as controls, were sent in a fresh condition. It was obvious that this procedure was not altogether satisfactory and in all the other experiments we arranged to have the stomachs sent to us perfectly fresh, so that there was the least possible delay between the time of killing and the commencement of their analysis. It must be further stated, however, that the above experiments are as perfectly reliable as the later experiments with fresh stomachs, which tended in all respects to confirm these first results.

(2) *Experiments made on Mice and Rats with Special Reference to Time of Digestion.*

It was obvious, however, that a definite period of digestion would be likely to give a more strictly comparable series of results, and we selected in the first instance a period of one hour. The mice, whether normal or with transplanted tumours, were put in cages overnight without food, were fed next morning and killed one hour after feeding.* Series 6 to 10 were conducted in this manner and a summary of results is given in Table B. The mice in this case, as in the previous series, were over twelve months old. The results obtained again appeared to emphasise, even more strongly, the fact that there was an average increase in the secretion of hydrochloric acid in the stomachs of mice with transplanted tumours as compared with normal mice during a period of digestion of one hour.

Thus, as against an average of 0·1488 per cent. in normal mice, we found an average of 0·1627 per cent. in mice with non-ulcerated tumours and of 0·2100 per cent. in mice with ulcerated tumours.

But a difficulty in these, as also in the previous experiments, was that although in the whole of the 10 series, with two exceptions only, a more or less considerable increase of hydrochloric acid was indicated in the case of the mice with transplanted tumours, the actual percentages of hydrochloric acid in each class varied nevertheless somewhat largely *inter se*, and more especially in the normal stomachs.

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* The food consisted of bread soaked in water, oats, and canary seed.

Table B.—Percentage of Physiologically Active Hydrochloric Acid in the Gastric Contents of Normal Mice, and of Mice with Transplanted Tumours. Period of digestion, 1 hour.

Series.	Date.	Normal mice.		Mice with non-ulcerated tumours.		Mice with ulcerated tumours.	
		No. of stomachs.	Hydrochloric acid.	No. of stomachs.	Hydrochloric acid.	No. of stomachs.	Hydrochloric acid.
6	May 30, 1906	32	0·1374	35	0·1393	21	0·2439
7	June 21, 1906	16	0·1992	15	0·1569	11	0·1898
8	Jan. 15, 1907	40	0·1188	50	0·1660	20	0·1856
9	Feb. 4, 1907	30	0·1322	45	0·1509*		
10	Feb. 28, 1907	26	0·1974	38	0·1964		
Average		144	0·1488	183	0·1627	52	0·2100

* These mice had been inoculated from Tumour "No. 27" (adeno-carcinoma); all the rest from alveolar carcinoma (Jensen). The weights of their tumours varied practically over the same range as given in note to Table A.

We had already considered the question as to whether it might be better to analyse the gastric contents apart from the very thin stomach walls, and we made a number of experiments in this direction. The following typical instance of such results in Series 8 (Table B) shows that the presence of the walls modifies but very slightly the results which would have been obtained for walls and contents taken together, and affects the comparison practically not at all.

——	Normal.	Non-ulcerated.	Ulcerated.
	HCl per cent.	HCl per cent.	HCl per cent.
Contents	0·1221	0·1783	0·1940
Walls	0·1061	0·1052	0·1357
Contents and walls	0·1188	0·1660	0·1856

We therefore decided to continue the experiments, as we had commenced them, by taking the walls and contents together.

We therefore next endeavoured by a further series of experiments to determine, if possible, the usual period of digestion of normal mice, so that this being done, we could then make further comparisons with mice with transplanted tumours under exactly similar conditions as to feeding and periods of digestion. We selected the following periods: half an hour, one hour (with mice under three months), and one hour and a-half.

Table C gives a summary of the results which we obtained and we have also included previous results for the sake of general comparison.

We found in the half hour's digestion, for mice over twelve months, the average in Series 12 was 0·1129 (10 stomachs), with a variation from the low figure 0·0611 to 0·2037 per cent., and in Series 16 the figures vary from 0·1243 to 0·1765 per cent., with an average of 0·1451 (7 stomachs). On the other hand, for mice under three months we obtained an average of 0·1881 per cent., with a variation from 0·1438 to 0·2167 per cent. (13 stomachs). The general average for all half hour experiments is 0·1530.

It seems to us, with this very considerable variation, scarcely worth while to continue the investigation of the half hour period.

Taking the one-hour period of digestion, the average results are more uniform, although there are considerable variations. The average for mice over twelve months (144 stomachs) being 0·1488, while that for mice under three months (55 stomachs) is 0·1445.

As regards the one-and-a-half-hour period, the average results were also very uniform and the variation distinctly less, the average for mice over twelve months being 0·1826 per cent., while that for mice under three months is 0·1866 per cent, the extreme variations being 0·1515 and 0·2643 per cent.

From these figures it is evident that no definite period of digestion is indicated from these experiments; all that can be said is that some mice have attained a maximum at half an hour, and most have attained a maximum at an hour and a-half, while at one hour a large proportion have attained a maximum. The explanation of this variation undoubtedly lies in the fact that mice are in the habit of continually feeding, and that therefore the intervals of rest from feeding are comparatively rare.

We next made further experiments as to the secretion of hydrochloric acid by mice with transplanted tumours during periods of digestion of one hour and one and a-half hours respectively. A summary of the results obtained is given in Table D, in which are also included our previous experiments, for the purpose of general comparison.

It will be seen from an inspection of Table D that the results for one hour's digestion of mice with non-ulcerated tumours (Series 6 to 10) gave an average of 0·1627 per cent. (183 stomachs), with a variation from 0·1393 to 0·1964 per cent.; while mice with ulcerated tumours (Series 6 to 8) gave an average of 0·2100 per cent., with a variation of 0·1856 to 0·2439 per cent.; the total average for these 235 stomachs was 0·1732 per cent., all the mice being over twelve months.

For mice under three months with transplanted tumours (Series 25 and 27), four gave an average of 0·1856 per cent. and seven an average of

0·1569 per cent., with a total average of 0·1673 and a variation from 0·1421 to 0·2102 per cent. during one hour's digestion.

With regard to the one-and-a-half hour period, 12 mice with transplanted tumours gave an average of 0·1605 per cent. (Series 22), 11 gave an average of 0·1260 per cent. (Series 23), and 11 gave an average of 0·1246 per cent. (Series 24), the general average being 0·1377, with a variation between 0·1013

Table C.—Percentages of Physiologically Active Hydrochloric Acid secreted by Normal Mice during Varying Periods of Digestion.

Period of Digestion, half an hour.					
(*a*) Over 12 months.				(*b*) Under 3 months.	
Series 12. Feb. 4, 1908.		Series 16. Feb. 11, 1908.		Series 14. Feb. 7, 1908.	
No. of stomachs.	Hydrochloric acid.	No. of stomachs.	Hydrochloric acid.	No. of stomachs.	Hydrochloric acid.
6	0·0611	2	0·1243	6	0·2167
1	0·1790	4	0·1476	4	0·1438
1	0·1817	1	0·1765	1	0·1788
1	0·1976			2	0·1962
1	0·2037				
10	0·1129 (av.)	7	0·1451 (av.)	13	0·1881 (av.)

Period of Digestion, one hour.							
(*a*) Over 12 months.		(*b*) Under 3 months.					
Series 6—10.* May 30, 1906—Feb. 28, 1907.		Series 19. March 4, 1908.		Series 20. March 11, 1908.		Series 21. March 17, 1908.	
No. of stomachs.	Hydrochloric acid.	No. of stomachs.	Hydrochloric acid.	No. of stomachs.	Hydrochloric acid.	No. of stomachs.	Hydrochloric acid.
32	0·1374	4	0·1353	12	0·1005	14	0·1260
16	0·1992	2	0·1654	4	0·2006	2	0·1397
40	0·1188	11	0·1686	2	0·2845	4	0·1508
30	0·1322						
26	0·1974						
144	0·1488 (av.)	17	0·1604 (av.)	18	0·1432 (av.)	20	0·1323 (av.)

* Repeated here from p. 449 for purposes of comparison.

Table C—*continued.*

Period of Digestion, one hour and a-half.							
(*a*) Over 12 months.				(*b*) Under 3 months.			
Series 11. Feb. 4, 1908.		Series 15. Feb. 11, 1908.		Series 13. Feb. 7, 1908.		Series 18. Feb. 25, 1908.	
No. of stomachs.	Hydrochloric acid.	No. of stomachs.	Hydrochloric acid.	No. of stomachs.	Hydrochloric acid.	No. of stomachs.	Hydrochloric acid.
6	0·1515	1	0·1575	6	0·2002	12	0·1716
1	0·2310	4	0·1673	2	0·1894		
1	0·2643	2	0·1684	1	0·1929		
		6	0·2110	4	0·2085		
8	0·1755 (av.)	13	0·1869 (av.)	13	0·2005 (av.)	12	0·1716 (av.)

and 0·1683. All these mice were under three months. The nature of the tumours is indicated in the note to Table D.

Apparently in the experiments relating to mice with transplanted tumours, the greater proportion have attained a maximum at the one-hour period of digestion.

Comparing the general results both for normal mice and for mice with transplanted tumours, it is obvious that there is no general tendency to a decrease in the latter as regards secretion of hydrochloric acid, the variations in both cases being strictly comparable, and, in fact, if we take the average of all mice examined in the two classes, we find that for periods of digestion of one hour and of one hour and a-half, 245 normal mice gave an average of 0·1546 per cent., while 290 mice with transplanted tumours during the same periods of digestion gave an average of 0·1673 per cent., that is a slight *increase.* Again, in the experiments made without reference to time of digestion (Table A), 150 normal mice gave an average of 0·1121, and 178 mice with transplanted tumours gave an average of 0·1752.

Only quite recently we have had the opportunity of examining a series of rat stomachs also supplied by Dr. Bashford, and as these varied in weight (after one hour's digestion) from 2·5 to over 10 grammes, it was a comparatively easy matter, and obviously advantageous, to analyse them singly.

We examined 13 such stomachs after one hour's digestion, three from rats which had been inoculated from tumours identified at the Imperial Cancer Research Laboratories as Flexnor 15B and 15A, and four from rats which had been inoculated from a tumour known as Jensen 8A.

Table D.—Percentage of Physiologically Active Hydrochloric Acid in Mice with Transplanted Tumours.

Period of Digestion, 1 hour.

(*a*) Over 12 months.				(*b*) Under 3 months.			
Series 6—10.* May 30, 1906—February 28, 1907 (non-ulcerated).		Series 6—8. May 30, 1906—January 15, 1907 (ulcerated).		Series 25. May 15, 1908.		Series 27. May 21, 1908.	
No. of stomachs.	Hydrochloric acid.	No. of stomachs.	Hydrochloric acid.	No. of stomachs.	Hydrochloric acid.	No. of stomachs.	Hydrochloric acid.
35	0·1393	21	0·2439	4	0·1856	1	0·2102
15	0·1569	11	0·1898			4	0·1421
50	0·1660	20	0·1856			1	0·1642
45	0·1509*					1	0·1553
38	0·1964						
183	0·1627 (av.)	52	0·2100 (av.)	4	0·1856 (av.)	7	0·1569 (av.)

* For tumours in Series 6—10 see notes to Table B. In series 25 the tumour was "No. 27" adeno-carcinoma, and in Series 27 squamous cell carcinoma.

Period of Digestion, 1½ hours.

Mice under 3 months.†

Series 22. May 5, 1908.		Series 23. May 6, 1908.		Series 24. May 9, 1908.	
No. of stomachs.	Hydrochloric acid.	No. of stomachs.	Hydrochloric acid.	No. of stomachs.	Hydrochloric acid.
6	0·1527	6	0·1290	5	0·1236
6	0·1683	2	0·1151	2	0·1218
		3	0·1272	2	0·1013
				2	0·1530
12	0·1605 (av.)	11	0·1260 (av.)	11	0·1246 (av.)

† In Series 22, the tumour was squamous cell carcinoma and alveolar-carcinoma (Jensen) respectively. In Series 23 they were all squamous cell carcinoma, and in Series 24 the first three were "No. 27" and in the last was alveolar carcinoma (Jensen).

These seven had all developed tumours ranging in weight from 0·3 to 15 grammes. The remaining six had been inoculated with the same tumours, but in all of them the result of such inoculation had been completely negative.

Table E shows a brief summary of the results obtained in both series.

Table E.—Percentages of Physiologically Active Hydrochloric Acid in Stomachs of Rats with Transplanted Tumours (positive), and in Normal Rats (negative); 1 hour's digestion.

Series 26. May 19, 1908.			
No. of stomachs.	Hydrochloric acid.	No. of stomachs.	Hydrochloric acid.
(*a*) Positive.		(*b*) Negative.	
1	0·1143	1	0·1192
1	0·2276	1	0·1076
1	0·2574	1	0·1400
1	0·1835	1	0·1358
1	0·1164	1	0·1564
1	0·2047	1	0·1975
1	0·1822		
7	0·1837 (av.)	6	0·1427 (av.)

It will be seen from the figures given in Table E that for one hour's digestion the rats with transplanted tumours gave an average of 0·1837 per cent. of hydrochloric acid, while those in which result of attempted transplantation had been negative, and which may therefore be regarded as normal, gave an average for the same period of digestion of 0·1427 per cent. The variations from the average are in neither series very considerable.

Finally, we may refer to the examination between May, 1906, and May, 1908, of 15 stomachs of mice affected with tumours which, though undoubtedly cancer, were of entirely spontaneous origin. For these, again, we are indebted to Dr. Bashford.

The following Table F gives a summary of the results obtained.

It will be seen from an inspection of this table that the amount of hydrochloric acid in the gastric contents of mice with spontaneous tumours varied (with one exception, viz., 0·0961 per cent., January 18, 1908) between 0·1372 and 0·2627, with a total average for 15 stomachs of 0·1929 per cent.

Conclusions.—Allowing for individual variations, so unavoidable in physiological experiments, it may fairly be argued that under exactly similar conditions of feeding and of periods of digestion there was evidently no general tendency to decrease in the secretion of hydrochloric acid by mice, either with transplanted or spontaneous tumours (and, so far as our

Table F.—Percentage of Physiologically Active Hydrochloric Acid in the Stomachs of Mice with Spontaneous Tumours.

Date.	No. of stomachs.	Hydrochloric acid.
Period of Digestion, uncertain.		
May 29, 1906………	1	0·2286
Nov. 4, 1907………	1	0·1847
Nov. 5, 1907………	1	0·1372
Nov. 8, 1907………	1	0·1652
Nov. 26, 1907………	4	0·2136
Jan. 18, 1908………	1	0·0961
Jan. 18, 1908………	1	0·1856
Period of Digestion, 1½ hours.		
Feb. 25, 1908………	1	0·2627
Period of Digestion, 1 hour.		
May 18, 1908………	1	0·2132
May 18, 1908………	1	0·1634
May 18, 1908………	1	0·2120
May 18, 1908………	1	0·1903
General average ………………………		0·1929

experiments go, the same would appear to be the case with rats), but on the contrary, we found a distinct tendency towards increase of the hydrochloric acid.

Consequently it would appear that the conclusion arrived at by Moore and others, that a marked diminution or even an absence of free hydrochloric acid occurs in the gastric contents in malignant disease of organs other than the stomach in human beings, is certainly not borne out by the results of our investigations on the amount of physiologically active hydrochloric acid present in the stomach contents of mice suffering from cancer.

(3) *Experiments made on the Human Subject. (Examination of Test-meals.)*

In the absence of determinations of hydrochloric acid in the gastric contents of man, by the same methods that we had employed in our work on mice, it did not seem possible to arrive at any satisfactory comparison of the results which we have set out in this paper, with those of Moore and his fellow-workers. This being so, it appeared to us to be a matter of importance to carry out a series of estimations on patients suffering from undoubtedly malignant disease, in situations other than the stomach, and, in addition, to determine the free hydrochloric acid and some other constituents by methods which had also been used by Moore. We therefore commenced in May, 1907, the chemical examination of a series of test-meals from patients suffering from cancer which, by the kindness of Dr. Lazarus-Barlow, of Middlesex Hospital, Dr. Murrell, of Westminster Hospital, and Mr. Leaf, of the Cancer Hospital, we were able to obtain from time to time. Since the above date we have examined 34 test-meals. The results obtained as regards physiologically active hydrochloric acid, inasmuch as they appear to confirm our conclusions with reference to mice stomachs and to contradict or materially modify the conclusions arrived at by Moore and others, will be of interest and, in fact, become a necessary adjunct to this research.

The test-meal given in all cases consisted of one pint of tea and a large round of toast. It was given as usual in the morning, fasting, and was withdrawn one hour after administration. Ewald, with whom the suggestion of test-meals originated, laid down as an essential condition that the test-meal should be withdrawn without the addition of water. Apparently, however, whether owing to difficulty of manipulation (which in some cases is considerable) or from a lack of appreciation of this fundamental rule, water is occasionally added by some operators to aid the withdrawal of the test-meal. We found that in the test-meals supplied to us, as a matter of fact, a number of the earlier ones had been thus diluted, and it was, in fact, due to the much larger quantity of hydrochloric acid found in some later test-meals (which proved on enquiry to have been undiluted), that our attention was especially drawn to this unforeseen contingency.

Thus in Cases 1 to 4 (Middlesex Hospital), the hydrochloric acid found was approximately 0·06 per cent., while in Case 5 (Westminster) we found nearly 0·35 per cent. Again, Case 6 (Middlesex) gave us 0·09 per cent., while Cases 7 to 9 (Cancer Hospital), gave 0·16, 0·22, and 0·18 per cent., respectively. On enquiry in connection with these greatly differing results we ascertained that in Cases 1 to 4 water had been used in order to aid the withdrawal of the test-meal. On the other hand we learnt that in Case 5 and in Cases 7 to 9 the test-meals had been withdrawn without the addition of water.

Owing to repeated assurances that it was not feasible, except in isolated cases, to obtain the original undiluted meal, we agreed to the suggestion in the first instance with one of the house surgeons at the Cancer Hospital, that 10 ounces only of water should be used to aid the withdrawal of the test-meal, and that if the whole of this amount was not required, the remainder should be added to the fluid withdrawn. The withdrawal of test-meals 11 to 23 was carried out in this manner, and the hydrochloric acid estimated therein was found to vary between 0·036 and 0·1059 per cent.

The only conclusion we could come to from the results of the analyses of test-meals where a known quantity of water had been used in the withdrawal, is that each and all of them represent a quantity of hydrochloric acid which must be considerably less than the actual quantity originally present, but how much less it is impossible to say. We therefore once more discussed the matter with Mr. Leaf, who fully appreciated the point, and we were able by the exertions of Mr. Allen, his house surgeon at the Cancer Hospital, to obtain nine further test-meals which had all been withdrawn without the addition of any water whatsoever. That the difficulty of obtaining undiluted test-meals is not exaggerated is indicated in a statement made to us by Mr. Allen, who says that on an average, where test-meals had been given, two out of three cases had to be abandoned owing to the impossibility of inducing the withdrawal of the fluid from the stomach of the patient.

Taking now the whole of the 34 test-meals from cancerous patients, we found in five of these in which the amount of water used in the withdrawal was unknown, the average amount of hydrochloric acid was 0·0646 per cent., with a minimum of 0·0552, and a maximum of 0·0895 ; in 16 cases in which the amount of water used in the withdrawal was known, the average amount of hydrochloric acid was 0·0554 per cent., with a minimum of 0·0127 per cent. and a maximum of 0·1059 per cent.

But in the 13 cases where the test-meals were withdrawn without the addition of any water whatever, in two instances only was the hydrochloric acid as low as 0·064 and 0·073 per cent.; in another exceptional instance it was as high as 0·35 per cent. (cancer of liver), while in the remainder it varied between 0·084 and 0·22, seven of these being over 0·16 per cent., with a total average in all 13 cases of 0·1626 per cent., as the following summary (Table G) will show.

As showing the influence of the addition of water in the withdrawal of the test-meal on the results obtained, the following figures will be of interest :—

Case 8 (Cancer Hospital, October 21, 1907), no water used. Found 0·2227 per cent. HCl.

Case 11 (same patient, October 30, 1907), 10 oz. water used. Found 0·0748 per cent. HCl.

Case 24 (Middlesex Hospital, January 28, 1908), 20 oz. water used. Found 0·0127 per cent. HCl.

Case 25 (same patient February 4, 1908), 5 oz. water used. Found 0·0858 per cent. HCl.

Table G.—Analyses of Gastric Contents (undiluted Test-meals) of Patients suffering from Cancer.

Case.	Date of receipt of test-meal.	Hospital.	Sex of patient.	Age.	Nature of disease.	Volume of stomach fluid obtained.	Günsburg test.	Total acidity to phenolphthalein reckoned as HCl.	Total chlorides.	Inorganic chlorides.	Hydrochloric acid (physiologically active).	Hydrochloric acid free and organically combined. (Mörner Sjöqvist.)	Hydrochloric acid (free) calculated from velocity of inversion of methyl acetate.	Organic acids reckoned as hydrochloric acid.	Total organic acids reckoned as hydrochloric acid.
Period of Digestion, 1 hour.															
5	1907 June 21	Westminster Hospital	F	61	Cancer of bile-duct	52 c.c.	Very marked	per cent. 0·2737	per cent. 0·3650	per cent. 0·0182	per cent. **0·3468**	per cent. 0·3525	per cent. 0·1940	per cent. Nil	per cent. 0·0036
7	Oct. 21	Cancer Hospital ...	F	62	Carcinoma of breast	21 c.c.	Marked	0·1825	0·2190	0·0547	**0·1643**	—	0·0753	0·0182	0·0182
8	Oct. 21	,, ,, ...	F	63	Carcinoma of breast	80 c.c.	Faint	0·2993	0·2482	0·0255	**0·2227**	0·1240	0·0305	0·0760	—
9	Oct. 22	,, ,, ...	F	40	Advanced carcinoma of cervix	35 c.c.	Nil	0·1642	0·2555	0·0730	**0·1825**	—	0·0074	Nil	0·0182
26	1908 March 10	,, ,, ...	F	59	Carcinoma of breast	105 c.c.	Nil	0·0839	0·2208	0·1095	**0·1113**	0·0564	0·0045	Nil	0·0255
27	March 13	,, ,, ...	F	50	Carcinoma of cervix (inoperable)	115 c.c.	0·0693	0·1971	0·2774	0·1149	**0·1625**	0·1566	0·0310	0·0346	0·0328
28	March 18	,, ,, ..	F	50	Carcinoma of cervix	137 c.c.	Nil	0·0474	0·1022	0·0382	**0·0639**	0·0376	0·0022	Nil	0·0073
29	March 27	,, ,, ...	F	34	Carcinoma of uterus (inoperable)	60 c.c.	Faint	0·1095	0·2044	0·0730	**0·1314**	0·1128	0·0072	Nil	0·0182
30	March 31	,, ,, ...	M	51	Cancer of stomach	210 c.c.	Faint	0·1606	0·1752	0·0912	**0·0840**	0·0125	0·0036	0·0766	0·1022
31	April 7	,, ,, ...	F	64	Carcinoma of uterus	112 c.c.	0·0803	0·2117	0·3467	0·1277	**0·2190**	0·2146	0·1076	Nil	0·0328
32	May 15	,, ,, ...	F	40	Carcinoma of uterus (recurrent)	65 c.c.	0·0305	0·1533	0·3212	0·1606	**0·1606**	0·1660	0·0401	Nil	0·0876
33	May 21	,, ,, ...	F	62	Carcinoma of breast	148 c.c.	Nil	0·0620	0·1350	0·0620	**0·0730**	0·0673	0·0061	Nil	0·0182
34	May 28	,, ,, ...	F	62	Carcinoma of breast (recurrent)	140 c.c.	0·0182	0·1825	0·2847	0·0931	**0·1916**	0·1848	0·0201	Nil	0·0328
							Average	0·1636	0·2427	0·0801	**0·1626**	0·1142	0·0407	—	0·0306
Normal Case.															
35	June 2	... ...	M	25	Normal ...	90 c.c.	0·1314	0·1861	0·3175	0·0839	**0·2336**	0·2068	0·1195	Nil	0·1004

As already said, we did not confine ourselves in the analysis of the test-meals to the estimation of those constituents only which we had determined in the case of the mice, viz., the total acidity, total chlorides and the inorganic chlorides (from which determinations we deduced the physiologically active hydrochloric acid and the free organic acids), but we also determined the free hydrochloric acid by the methyl acetate process, the free and organically combined hydrochloric acid by the Mörner Sjöqvist process, and also the total organic acids, free and combined, all of these further determinations having been made by Moore in his analyses of test-meals.* We also applied the Günsburg test in all cases, and where a very marked reaction was obtained we also made the test quantitatively.

We will now briefly discuss the results obtained in these estimations.

We have shown above that in 13 test-meals which had been withdrawn without any addition of water whatever, we obtained an average of 0·1626 per cent. physiologically active hydrochloric acid; five of them being above 0·18 per cent. All of these test-meals were obtained from cases of undoubted cancer. The standard amount of hydrochloric acid in the gastric juice is usually quoted as 0·2 per cent., in healthy individuals, and this has been more or less generally accepted, apparently, without any very definite basis of authority. Moore states in his paper of March, 1905, read before this Society, that in aged individuals the free hydrochloric acid is said to be only slightly over 0·1 per cent., in which case the average we find of physiologically active hydrochloric acid (obviously free at some time during the process of digestion), is well over normal, inasmuch as seven out of the 13 test-meals examined were obtained from individuals between 59 and 64 years of age. As regards our estimations of free hydrochloric acid by the velocity of inversion of methyl acetate, which we carried out in the manner referred to by Moore, we obtained an average of 0·0407 per cent., in the 13 undiluted test-meals from cancer patients, with a variation between 0·0022 per cent. and 0·1940 per cent., all the meals having been withdrawn exactly one hour after administration. Moore, in his first paper already alluded to, quotes 12 cancer cases with an average of 0·0039 per cent. of free hydrochloric acid, but in his second paper (May 9, 1906) in the 'Bio-chemical Journal,' vol. 1, p. 274, he quotes 13 additional cases with an average of 0·0515 per cent. free hydrochloric acid, or about 13 times as much as in the previous instances. It certainly seems curious that in two series of cases of typical carcinoma there should be this remarkable difference, and the fact does not suggest that the determination of free hydrochloric acid is of any diagnostic value, or, as far as we can see, of any practical importance, and it is a striking fact that in 20 hospital cases (non-malignant), Moore found an average of 0·063 per cent. of free hydrochloric acid, an amount which differs but slightly from the average of his second series of cancer cases (0·051 per cent.). Dr. Palmer, employing the same method, estimated the free hydrochloric acid in test-meals from 14 cancer patients, and found an average of 0·0217 per cent. It may be noted that Palmer lays special stress on the necessity of obtaining the withdrawal of the test-meal without any possibility of dilution with water. Moore makes no reference to this point, but in Case 9 states, that instead of 1 pint of tea, 1 pint of gruel and 2 pints of water were administered and withdrawn one and a-half hours afterwards. It is, perhaps, hardly surprising that, under these circumstances, the free hydrochloric acid obtained was only 0·001 per cent.

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* Ten cubic centimetres of filtered gastric contents were invariably taken for these estimations.

It is of importance to note, also, that the interval between the administration of the test-meals and its withdrawal, as recorded by these observers, varied between the wide ranges of one to two hours, and in a single case was as much as three hours. This absence of uniformity as regards time of withdrawal clearly detracts from the diagnostic value of the results, and it is sufficiently obvious that where a number of experiments are being made under varying conditions of time, whether by one or more observers, no proper comparison will be possible between the results or the deductions drawn from them.

We may next discuss the results of the Mörner Sjöqvist estimations. This method is intended to estimate both the free hydrochloric acid and that combined with proteid or organic bases. The underlying principle is that, on bringing the filtered gastric contents into contact with pure barium carbonate, these various forms of hydrochloric acid interact with it to produce barium chloride, and that, on subsequent evaporation, incineration, and extraction with water, the amount of barium chloride formed may be estimated as barium sulphate and expressed in terms of the original hydrochloric acid. In 16 out of 34 test-meals examined, the result obtained by this method agreed almost theoretically with the physiologically active hydrochloric acid found by the method of precipitation with silver; in the remainder it was less, in some cases considerably so. The failure to estimate the full amount of hydrochloric acid in these cases we account for by the presence of mucus-like substances in the fluid examined which, by preventing complete contact of the solid barium carbonate, causes loss of hydrochloric acid during evaporation. The fact, however, that in several cases the method gave results strictly comparable with our silver determinations, entirely disposes of the objection raised by Moore that the method is liable to estimate other inorganic acids than hydrochloric, which he suggests might be present. He makes, however, no statement as to the particular inorganic acids intended. Inasmuch as phosphate and sulphate of barium are insoluble salts, there only remains nitric acid, the barium salt of which is decomposed on ignition. In our opinion, this Mörner Sjöqvist method is fairly accurate, but that it is not applicable in certain cases is shown above, and as there are other objections to it, not the least of which is the length of time it requires, we do not see any advantage in its employment, since Volhard's method amply suffices. Neither Palmer, nor Moore, in his second paper, make any allusion to the work of Lüttke or Willcox, in which Volhard's method was employed.

With reference to the estimation of the organic acids, free and combined, we have made estimations in all cases by the method used by Moore, and the average result in 12 undiluted test-meals was 0·0331 per cent., with a variation from 0·0036 to 0·1022 per cent. Here, again, we differ from Moore, whose results are invariably low. We obtained, from our own determinations referred to above, the figures for free organic acids by difference, but in all but four cases they were entirely absent.

So much has been written of late with reference to the Günsburg reaction that it is unnecessary to enter into any detail here. It will therefore suffice to say, as regards our own experience, that in only a very few cases in all the test-meals examined, even those which had been diluted, did we fail to get a more or less marked reaction, and in the five cases in which the test was made quantitatively, three of the results approximated to the figure obtained by the methyl acetate process, while two were distinctly higher.

Conclusions.—It would appear, therefore, from our experiments on test-meals from cancer patients, that the physiologically active hydrochloric acid, although appearing in a few cases to be somewhat low, can, in the majority of instances, be regarded as normal, or even above normal, according to the standard of hydrochloric acid accepted as normal. It may be that possibly the free hydrochloric acid in the gastric contents is liable to be diminished in disease, whether malignant or not; but unless the experiments are made under rigid precautions as to avoidance of dilution in withdrawal of the test-meals, and unless the observations made by different observers have reference to exactly the same period of digestion, any comparison of results and any deductions from them must tend to be fallacious. Moreover, as already shown, free hydrochloric acid in the gastric contents may at any moment become combined with proteids and nitrogenous organic bases, and it is obvious that the rate of secretion of hydrochloric acid in the gastric juice, inasmuch as it varies in healthy individuals, may also be expected to vary under conditions of disease, and if this be so, then another element of uncertainty is introduced into its determination, however rigid the conditions of the experiment may be.

Dr. Bashford has stated* that on the basis of observations made on almost 3000 mice with propagated cancerous tumours during two years, "the conclusion has been arrived at that the presence of a tumour even of greater weight than the mouse itself does not necessarily involve a disturbance of the normal nutrition which could be regarded as comparable to the cachexia frequently associated with malignant new growths in the human subject."

But inasmuch as recent extensive statistics, collected by the Imperial Cancer Research Fund† from various London hospitals, have shown that cachexia is not a constant accompaniment of cancer in man, might we not expect to find the same compensating influence as regards increased or undiminished secretion of hydrochloric acid in the stomachs of human beings affected with this disease?

It is certainly clear from our experiments, so far as they go, that the percentage of physiologically active hydrochloric acid in the gastric contents of mice and rats is, on the whole, slightly greater when they are the subject of cancerous growths as compared with these animals under normal conditions; and although we cannot, of course, from the comparatively few experiments made by us on the human subject, assert that the secretion of hydrochloric acid in cancer is normal (for in a few cases it was decidedly below normal), yet we think that we have sufficiently indicated that the whole question of the amount of hydrochloric acid in human gastric contents in cancer would repay further investigation, and that it is not justifiable, in the absence of more comparable experiments than those at present available, to conclude that it is always greatly diminished in the presence of this disease.

In conclusion, our thanks are due to Mr. S. Bosworth for his able assistance, throughout the whole of this work, in the details of the numerous chemical estimations involved.

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* 'Scientific Reports on the Investigations of the Imperial Cancer Research Fund,' No. 2, 1905, p. 40.

† 'Transactions of the Epidemiological Society of London' (N.S.), vol. 26, 1906—7.

The Giant Nerve Cells and Fibres of Halla parthenopeia.

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(Communicated by Professor J. C. Ewart, M.D., F.R.S. Received May 30,—Read June 18, 1908.)

(Abstract.)

An anterior and a posterior series of giant cells are present in *Halla parthenopeia*; the following statement refers to the anterior series.

The primary giant cells are formed in segmental couples—one couple in each of the anterior ganglia of the nerve cord—until a maximum of eight couples is attained. While the last three couples are being formed, secondary giant cells are also formed at the anterior end of the nerve cord and occasionally in one or more ganglia already possessing a primary couple The number of giant cells in full-grown specimens is usually 15 to 18, but specimens with 20 and 21 are recorded.

There is a progressive increase in the size of the primary giant cells until the worm has attained a length of 30 to 40 cm., by which time the giant cells appear to have reached their maximum size. The largest cells are usually those of the second and third couples, which, in adult worms, are 130 to 150 μ in diameter.

Yellow granules are present in the giant (and in some of the larger ganglion) cells, the substance and pigment of which are closely similar to, if not identical with, those of the chlorogogen granules. The granules in the nerve cells therefore appear to be insoluble products of metabolism.

Small, rounded, chromophilous granules are present in the protoplasm (except in a peripheral zone, in which they are almost or quite absent) in varying amount in different giant cells. They are found in greatly increased mass in a specialised peri-nuclear zone, which is distinguishable in the living cell by its greater refringency. The outer edge of this zone is bounded by the peri-nuclear network of neurofibrillæ, which is thus in a position which facilitates its rapid nutrition. In many of the cells there is a "double nucleolus" which consists of one or more acidophile bodies enveloped by a basophile substance.

Each giant fibre, after leaving the giant cell from which it arises, crosses the cord to the opposite side, turns gradually towards the middle line of the cord and runs posteriorly. Some of the largest fibres, from two to six in different specimens, run to within 1 or 2 mm. of the posterior end of the worm, the other fibres taper and successively disappear after running various

distances down the cord. One or more short branches issue from the giant fibre near the angle of decussation, and, as the fibre runs along the dorsal side of the nerve cord, branches issue which fork, the twigs pass to the right and left, they taper, their sheath disappears, and the protoplasmic axis of each twig, now about 1 μ in diameter, is lost to view in the lateral or ventro-lateral region of the neuropile, in no case could it be traced into a spinal nerve.

The sheath of the giant cell and giant fibre is not blackened by the action of osmic acid, it consists of glia fibrils of various thickness, among which glia nuclei and the granular remains of glia protoplasm are present.

The neurofibrillar network in the giant cell is divisible into a peri-nuclear network, situated at the margin of the peri-nuclear zone, and a more extensive, wider meshed, and generally more slender stranded network in the general protoplasm. From this network slender primitive fibrils pass into the cone of origin of the axone, whence stouter fibrils (0·2 to 0·25 μ thick), each due to the fusion of several primitive fibrils, pass into the giant fibre. The number of fibrillæ issuing from the cell varies according to the size of the cell: 6 to 10 issue from the small giant cells, 12 to 30 from the larger ones. The bundle of neurofibrillæ probably does not fill the lumen of the fibre in life (fibrillæ could not be seen in the living or fresh giant fibres), but occupies from one-fourth to three-fourths of the internal diameter of the fibre, the remaining space being filled with the semi-fluid, finely-granular "peri-fibrillar substance." Between the fibrils there is a more homogeneous "inter-fibrillar substance." The fibrillæ in a giant fibre are usually all of the same thickness, but in several fibres there are one to three fibrils thicker than the rest.

The contents of the giant fibre are equivalent, and have a similar structure, to the axis cylinder of a medullated nerve, except that in the former there is nothing comparable to the Ranvier's nodes of the latter.

The anterior giant cells of *Aglaurides fulgida* are also segmentally arranged, there being a couple in each of the first four, five, or six segments. The cells seem to have attained their maximum diameter (90 μ) when the worm has reached a length of 14 cm. In the main features of their structure and of the arrangement of their neurofibrillar network the giant cells of *Aglaurides* agree with those of *Halla.*

Sharply and deeply stained fibrils penetrate the sheath of many of the giant cells of *Halla* and *Aglaurides,* enter the cell and apparently join the intracellular network. Numerous short fibrils, probably of glial nature, enter the peripheral zone of the giant cells of *Halla.*

Preliminary Account of the Habits and Structure of the Anaspididæ, with Remarks on some other Fresh-water Crustacea from Tasmania.

By Geoffrey W. Smith, M.A., Fellow of New College, Oxford.

(Communicated by Professor E. B. Poulton, F.R.S. Received June 10,—Read June 25, 1908.)

[Plate 13.]

In 1893, Mr. G. M. Thomson* described an interesting Schizopod from a small pool near the summit of Mount Wellington, which he named *Anaspides tasmaniæ*, and pointed out many peculiarities in its organisation, tending to show the primitive character of the animal.

Dr. W. J. Calman,† in 1897, revised Thomson's description and instituted a comparison between Anaspides and certain Carboniferous shrimps of Europe and North America (Gampsonyx, Palæocaris, etc.), and, in a later paper dealing with the classification of the Malacostraca as a whole, this author proposes to do away with the order Schizopoda altogether, and to redistribute its component families, uniting the Mysidacea with the Amphipoda, Isopoda, and Cumacea in a division, Peracarida, placing the Euphausiacea with the Decapoda in a division, Eucarida, while Anaspides constitutes a division, Syncarida, by itself.‡

The chief distinguishing features of the Peracarida are the incomplete nature of the carapace, the presence of a brood pouch in the female, formed from oostegites on the thoracic limbs, the elongated heart, the few and simple hepatic cœca, filiform spermatozoa, and direct development without complicated metamorphoses. The Eucarida show the exact converse of these characters.

From what was known of Anaspides, it seemed doubtful if it could be placed in either of these sub-classes, since although it was without a carapace, it appeared to possess no trace of a brood pouch. Quite recently, Mr. O. A. Sayce§ has described a remarkable fresh-water crustacean from the neighbourhood of Melbourne, which is evidently closely allied to Anaspides in its general structure, but it presents the curious feature of possessing sessile eyes, a character which makes it all the more difficult to preserve the Schizopoda as a natural group.

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* 'Linn. Soc. Trans.,' 2nd ser., vol. 6, 1894—97, Part 3, August, 1894, pp. 285—303, Plates 24—26.

† 'Roy. Soc. Edin. Trans.,' vol. 38, Part 4, 1896, pp. 787—802, Plates 1 and 2.

‡ 'Ann. Mag. Nat. Hist.,' 7th ser., vol. 13, 1904, p. 144.

§ 'Victorian Naturalist,' vol. 24, p. 117, 1907.

The observations which I have to offer upon the Tasmanian Anaspididæ both in respect of their habits in a natural state and of their anatomy, will, I believe, confirm the desirability of following Calman's classification in doing away with the Schizopoda as a natural group, and certainly of placing the Anaspidacea in a separate division.

I have also to report the discovery of a new genus and species of the Anaspididæ from the Great Lake of Tasmania, which I propose to name *Paranaspides lacustris,* so that at the present time, not counting possible fossil allies, three types of the Anaspidacea are known, viz., *Anaspides tasmaniæ* (Thomson) and *Paranaspides lacustris* (Smith), from Tasmania, constituting the family Anaspididæ, and *Koonunga cursor* (Sayce), representing a separate family, Koonungidæ, from southern Victoria.

Anaspides tasmaniæ (Thomson).

Habits and Occurrence.—During an expedition to Tasmania which I was enabled to undertake through the assistance of Professor G. C. Bourne and the British Association, four localities were established where the shrimp occurred: (1) in isolated pools and in the pools of the upper part of the North-west Bay River as it leaves the table-land on the top of Mount Wellington; (2) some large tarns near the summit of the Harz Mountains to the south-west of Hobart; (3) the tarns and mountain streams near the top of Mount Field to the north-west of Hobart; (4) in the tarns near Mount Read on the west coast. The animal, therefore, appears to be confined to the southern and western part of the island, and it is always found at a high elevation of 2000—4000 feet, in ice-cold water of absolute clarity.

The body of a very large specimen may measure 2 inches in length, and the yellowish skin is deeply pigmented with black chromatophors. There is no calcification in the exoskeleton, though small deposits of lime are present round the thoracic ganglia.

The animal pursues a creeping habit, walking about on the rocks and among the vegetable growths at the bottom of the pools; occasionally it swims lazily in the water, and on reaching the surface may turn over on its back, like a Phyllopod. When disturbed, it gives rapid side-strokes with the abdomen and tail fan, which drive it *forwards* or sideways; it never springs backwards, as so many Malacostraca do. The most interesting point about the creature in its natural state is that the body is held quite flat and unflexed, with the walking thoracic legs and abdominal pleopods, which also assist the walking movement, spreading out laterally from the body in the position shown in fig. 1 (Plate 13).

This unflexed posture, besides giving a very unshrimp-like appearance to the animal, is important, because the Carboniferous fossils referred to above are generally preserved in this condition, showing that they probably followed a similar habit of life.

Anaspides appear to be omnivorous, as they will feed upon the dead bodies of insect larvæ or even upon one another; but their chief food is the algal slime covering the rocks among which they live, and they also browse upon the shoots of submerged mosses and liverworts.

There is a point in regard to the function of the exopodites of the thoracic limbs which can only be observed in the living animal, and has been missed by previous authors; these exopodites are not locomotory in function, but are kept in a continual waving motion even when the animal is stationary, and serve to keep up a current of fresh water round the gills. Their function appears to be entirely respiratory.

All the numerous specimens observed appeared to be entirely free from any parasites, and the only native fish inhabiting the waters where it occurs is the little Mountain Trout (*Galaxias truttaceus*), which is probably not a formidable enemy. The introduced English trout, however, which are multiplying so rapidly in the Tasmanian rivers and lakes will probably end by exterminating the shrimp.

Anatomy.—There is very little to add to the descriptions that have been given of the external anatomy and form of the appendages, except in relation to the genital openings, which were erroneously described by Thomson. Both the male and female openings are in the normal Malacostracan position, *i.e.*, the vasa deferentia open at the bases of the last pair of thoracic legs, and the oviducts at the bases of the last pair but two. The large median opening on the ventral surface of the last thoracic segment in the female is not, as Thomson supposed, the aperture of the oviducts, but opens into a blind pouch, the spermatheca, where the male deposits the spermatozoa. This spermatheca is unparalleled in other "Schizopoda," and seems to point to Decapodan affinities.

The internal anatomy, studied in fresh material and by means of serial sections, reveals several points of importance.

The *heart*, which is tubular and elongated, stretches through the whole of the thorax, and passes without a very definite constriction into the abdomen. The ostia, of which there are apparently only a single pair, are situated in a constriction in the third thoracic segment. The structure of the heart recalls very closely that of the Mysidacea.

The *alimentary canal* is furnished with a gastric mill of a simple character, provided with numerous ridges and setæ; behind the stomach enter about thirty long, slender, and unbranched cœca of a glandular nature ("liver"). In the abdominal region, two dorsal unpaired cœca of a small size are present, which are difficult to compare with similar structures in the Decapoda, owing to the backward position of the anteriorly placed cœcum. The whole structure of the alimentary canal is peculiar, and not quite like that of any other group of the Crustacea.

The *ovaries* and *testes* are paired tubes stretching the whole length of the body. Their ducts are simple tubes not provided with accessory glands. The adult spermatozoa are filiform, with globular heads and an elongated flagellum, an important diagnostic character shared in common with the Mysidacea.

The *excretory glands* are large coiled tubes ending in an expanded sac and opening at the bases of the second maxillæ. No antennary glands are present.

The presence of maxillary glands, which are characteristic of the Entomostraca, must be looked upon as a primitive character; but it occurs in a few instances among the Isopoda as well. The *nerve-cord* consists of eight free thoracic ganglia, corresponding to the eight free thoracic segments, and six abdominal ganglia. The definite discovery that there is a free thoracic ganglion corresponding to the first thoracic segment is of some importance, since doubt has been thrown upon the real segmental value of this segment, which in all other Malacostraca is definitely fused with the head.

Breeding Habits.—Since there is no trace of a brood pouch, it was of interest to establish what the female does with her eggs, especially as this is a character of great taxonomic importance. The male deposits two very large spermatophors in the spermatheca of the female; these spermatophors are curved structures, and project outside the spermatheca. The spermatozoa pass into the spermatheca, and the spermatophors drop off. The eggs, which are pale purple in colour and measure about a millimetre in diameter, are passed out of the oviducts, and probably are fertilised as they pass out by spermatozoa, which migrate from the spermatheca, and possibly they are assisted in this migration by the setose projections on the internal faces of the coxopodites of the last three pairs of thoracic appendages, these projections being a sexual character confined to the female.

The female deposits and hides her eggs singly, and not agglutinated together, under stones and among the roots of water plants, being the only crustacean with the exception of the peculiar parasitic Argulidæ to do this.*

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* Certain Euphausiidæ also deposit their eggs, but not the family as a whole.

This habit of depositing the eggs, which separates Anaspides from all the other groups of the Malacostraca, is probably primitive, and it emphasises very strongly the isolation of the Anaspidacea from the other Malacostraca, all of which have the most constant and elaborate way of carrying the eggs, involving radical modifications of structure, none of which the Anaspidacea possess.

I was unable to observe the hatching out of the eggs, but the whole of the evidence I could bring together combines to show that the young hatch out with the essential if not identical structure of the adult.

Systematic Position.—The characteristics of Anaspides may be summarised in the form of a table setting forth those characters in which it is peculiar, and those in which it agrees with the other divisions of the Malacostraca in separate columns:—

Peracaridan. (Mysidacean.)	Eucaridan. (Decapoda.)	Peculiar features.
Absence of carapace. Structure of heart. Filiform spermatozoa.	Auditory organ on first antennæ. Modification of endopodites of first two pleopods as copulatory organs, and presence of spermatheca. Absence of lacinia mobilis on mandible.	Eight free thoracic segments with eight ganglia corresponding. Oviposition. Maxillary gland. Structure of alimentary canal. Plate-like structure of the double series of gills.

This table will serve to demonstrate the impossibility of including Anaspides in either the Peracarida or Eucarida, and since to include it in the old group Schizopoda would further stretch the bounds of that already heterogeneous collection, it seems desirable to adopt Calman's term, Syncarida, for a division to include Anaspides and its allies, both living and extinct.

Those characters which Anaspides does not possess peculiar to itself are shared either with the Mysidacea or else with the Decapoda, so that I am unable to follow Thomson and Sayce in finding special Euphausiid affinities. The resemblance to the Euphausiacea only concerns details in the structure of the appendages and cannot compare in importance with such characters as the elongated heart and the filiform spermatozoa which are actually diagnostic of the Mysidacea. The fact that the Anaspidacea seem to skip over the Euphausiacea and to link themselves more directly to the Decapoda would suggest that the Euphausiacea do not stand in the direct line of Decapodan descent, but have been secondarily derived from a primitive Decapod type by the loss of certain important characters, *e.g.*, the auditory organs on the second antennæ.

Paranaspides lacustris.

This type of a new genus of the Anaspididæ was found by me in the Great Lake on the central plateau of Tasmania at an elevation of 3700 feet. It inhabits the littoral zone of the lake, living among the rocks and water-weeds rather after the manner of a prawn. It is totally different in external appearance to Anaspides, being of a green transparent colour sparsely powdered with black dots, and the body exhibits a marked dorsal flexure very much as in Mysis, to which it bears an extraordinary superficial resemblance (fig. 2, Plate 13).

The largest specimen obtained was about an inch in length. It pursues a swimming habit, with which is correlated the flexure of the body, the elongation of the abdomen, the enlarged tail fan, and the enlarged scales on the second antennæ (figs. 3 and 4).

Fig. 3.

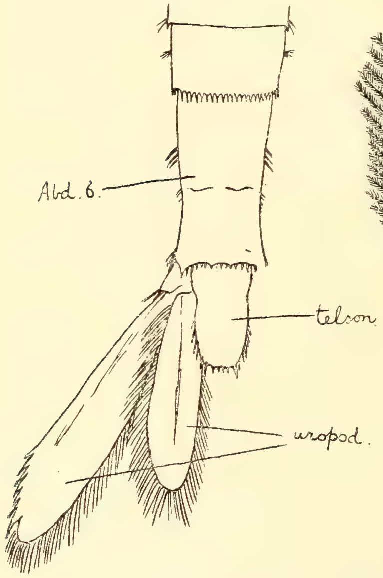

Fig. 4.

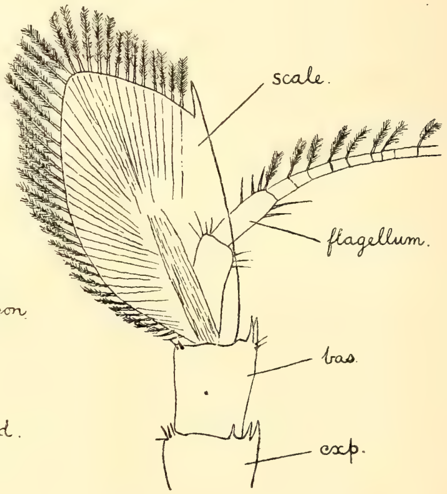

Fig. 3.—*Paranaspides lacustris.* Hind part of body, dorsal view. *Abd.* 6, sixth abdominal segment.

Fig. 4.—*Paranaspides lacustris.* Second antenna, dorsal view ; *bas.*, basipodite ; *cxp.*, coxopodite.

Besides these characters distinguishing it from Anaspides, important differences exist in the structure of the mandible, which bears a four-jointed and distinctly biramous palp (fig. 5), a characteristic only found elsewhere in Crustacea among the Copepoda, while the first thoracic appendage bears on the inner face of the antepenultimate joint a setose lobe used in mastication (fig. 6).

Fig. 5.

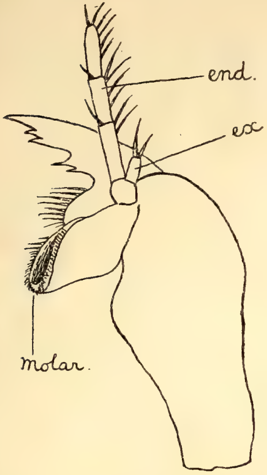

Fig. 6.

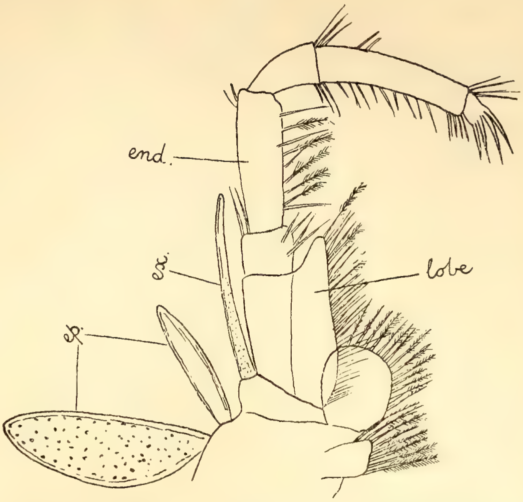

Fig. 5.—*Paranaspides lacustris.* Left mandible, inner view. *end.*, endopodite of palp; *ex.*, exopodite.

Fig. 6.—*Paranaspides lacustris.* First thoracic appendage. *end.*, endopodite; *ex.*, exopodite; *ep.*, epipodites; *lobe*, additional lobe on ischiopodite.

In all other essentials the structure agrees with that of Anaspides.

Associated with *Paranaspides lacustris* in the Great Lake was a very abundant development of ground-living Crustacea, including several Amphipods (Neoniphargus and Chiltonia), and three or four apparently new species of the peculiar Australian and New Zealand genus Phreatoicus, curious Isopods of a very generalised nature. One of these species was a handsome spiny form with orange-coloured antennæ and limbs, attaining about an inch in length.

The extremely abundant development of this Crustacean fauna in the littoral zone of the Great Lake was very striking, and it seems probable that it furnishes an important part of the food for the imported English brown trout, which in this lake may attain to the enormous weight of 25 lbs. A dissection of the stomachs of several trout revealed the fact that they had been feeding upon these creatures.

The Anaspidacea and Phreatoicidæ of Southern Australia and Tasmania really stand in much the same relation to other Crustacea as the Monotremata do to normal mammals, and it is of interest to enquire whence these peculiar Crustacea have been derived, and how it comes about that they are now restricted to this isolated corner of the Antipodes. The most striking thing about their distribution at the present time is the fact that not only are they absolutely confined to the temperate parts of the Australasian region, but also to the coldest parts of the temperate zone, the majority of the Anaspidacea and Phreatoicidæ being found at considerable elevations on mountain ranges which are covered with snow for at any rate a great part of the winter. They are absolutely unknown from Australia north of the Dividing Range.

A great deal of evidence has accumulated in recent years supporting the theory of the existence, perhaps in very early Tertiary times, of an Antarctic continent, affording means of communication between Southern Australia, South America, and to a less extent with New Zealand, upon which the characteristically temperate part of the fauna and flora now found, with many elements shared in common in these countries, was largely developed. The greatest community exists now between temperate Southern Australia and South America, the connection with New Zealand being apparently more ancient.

If this Antarctic Continent really existed, it is fairly certain that the Anaspidacea and Phreatoicidæ, being not only temperate but alpine in their habitat, formed a part of its fauna; we should therefore be justified in expecting the discovery of these creatures or nearly related forms in temperate South America, especially as the Phreatoicidæ have already been found in New Zealand.

[*Note added while the paper was passing through the press.*—Certain other Crustacea that were commonly met with in the fresh-waters of Tasmania, especially in the highlands, have closely related forms in New Zealand and South America. Thus the Amphipod *Chiltonia australis* is exceedingly common in Tasmania, the genus being represented in South Victoria and New Zealand, and replaced in South America by the closely-related *Hyalella*. The commonest element in the plankton of the lakes and tarns of Tasmania is afforded by various species of the Copepod genus *Bœckella*, which entirely replaces the northern *Diaptomus*. *Bœckella* occurs again in New Zealand and temperate South America, but is unknown in the northern hemisphere. Other species of fresh-water Crustacea are closely related to forms in the northern hemisphere, but it seems very probable that they have reached Tasmania and Southern Australia *viâ* South America and the Antarctic continent. Such are the genera *Neoniphargus* and *Gammarus*, which are unknown in the tropics, and confined in their distribution to the temperate northern hemisphere and to temperate Australasia. The small planktonic genus *Bosmina*, which is widely distributed over the northern hemisphere and descends right down the Andes to temperate South America, was found by me as a common constituent of the plankton of the highland lakes in Tasmania. The genus

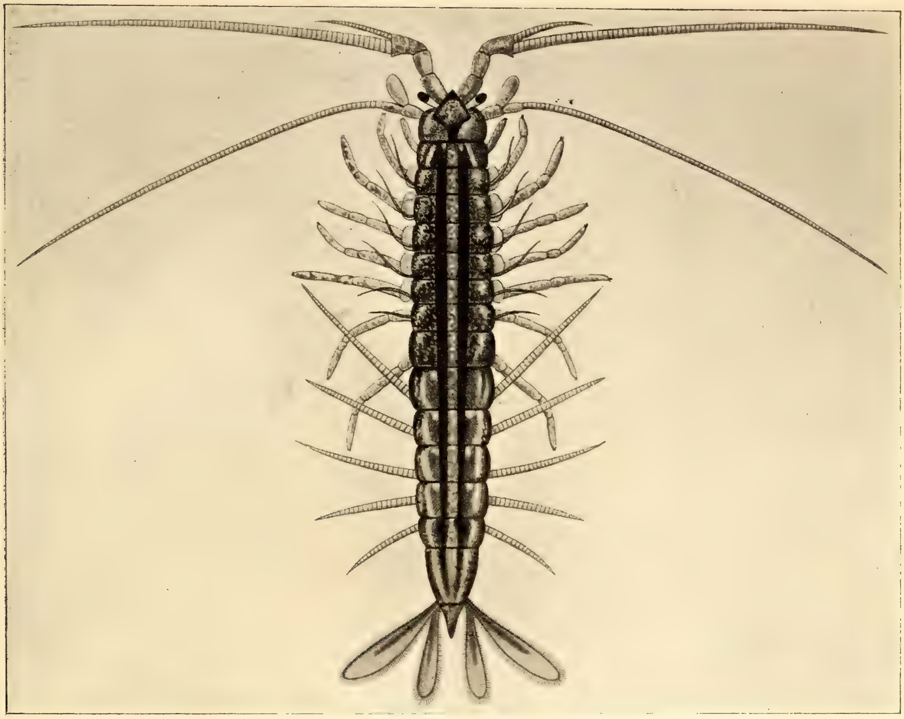

FIG. 1.—*Anaspides tasmaniæ.* Nat. size.

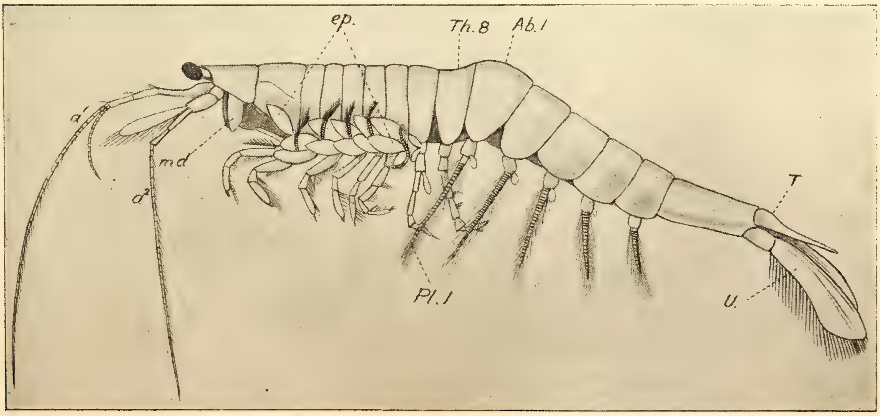

FIG. 2.—*Paranaspides lacustris.* × 4. a^1, 1st antenna ; a^2, 2nd antenna ; *md.*, mandible ; *ep.*, epipodites ; *Th.* 8, 8th thoracic segment ; *Ab.* 1, 1st abdominal segment ; *Pl.* 1, 1st pleopod ; *T.*, telson ; *U.*, uropod.

is quite unknown from the northern parts of Australia or tropical Asia, so that it would appear to have reached Tasmania *viâ* South America. The entire absence of a lofty mountain range like the Andes, running north and south through the tropics of the eastern hemisphere, has precluded the possibility of a temperate fauna from the north reaching temperate Southern Australia by that route.]

Some Experiments made to Test the Action of Extract of Adrenal Cortex.

By S. G. Shattock and C. G. Seligmann.*

(Communicated by John Rose Bradford, For. Sec. R.S. Received July 15, 1908.)

In a recent communication, in which we discussed the significance of secondary sexual characters in the Fowl, we suggested that certain of these characters, *e.g.*, the growth of spurs, "commonly regarded as essentially male, are not attributable to the function of the testicle alone, but possibly indicate the concurrent action of some other gland, perhaps the adrenal, seeing that not a few examples of precocious puberty in children have been found associated with adenomatous or carcinomatous growths of the adrenal gland."†

We selected the cortex for experiment because the new growths occurring in these cases consist of cortical tissue, and because so little is known of the physiological action of the cortex that there was nothing to negative the possibility referred to. The primary object of our experiments was to determine whether the injection of cortical extracts into young animals would hasten the appearance of secondary sexual characters, or exert any influence on the testicle. Since, however, there was no domestic animal smaller than the sheep, in which the male possesses well marked external secondary sexual characters, we were reduced to experiment on birds, selecting the common wild duck on account of the marked difference of plumage in the two sexes and the ease with which the bird can be kept in captivity.

Besides the gradual passage from its nestling to its adult winter and breeding plumage, the male of this species presents a seasonal change.

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* The expenses of this research were defrayed by a grant from the Government Grant Committee of the Royal Society.

† "An Example of True Hermaphroditism in the Domestic Fowl," 'Trans. Path. Soc.,' vol. 57, 1906, p. 109. The reason for excluding the testicle in this connection is that the growth of spurs is not inhibited by castration of the young bird.

After the breeding season, that is to say about the end of June, the vividly differentiated plumage of the drake gradually passes into the dusky summer or "eclipse" plumage which renders the drake remarkably like the female bird in the colour of its feathers; this "eclipse" should be complete by about the beginning of August, and after three weeks or a month the reverse change begins to be obvious, the bird gradually reassuming the typical male winter plumage. We did not limit our experiments to the injection of cortical extract into young birds, but we also experimented on birds approaching the "eclipse," and in "eclipse" plumage. The validity of our attempts, however, is marred by the fact that the injections invariably lead to a local necrosis of tissue, which, although it does not obviously affect the health or comfort of the bird, actually produces a delay in the appearance of the secondary sexual characters. The ideal experiment would doubtless be to inject young mammals with the extract from the cortex of adults of the same species; we have already explained why we were forced to employ birds in our experiments. The further departure from the ideal experiment occasioned by the substitution of an extract of mammalian cortex for that of the avian adrenal was necessary for the following reason:—In birds the cortex and medulla of the adrenal gland do not form well differentiated structures as in mammals, but strands of cortical and medullary cells are interwoven throughout the gland in such a way as to make it quite impossible to isolate one from the other. The glands used for preparing the cortical extract were at first procured from goats and used almost directly they were taken from the body, but later on sheep were substituted, and the glands were often kept upon ice for 12 hours before using them; we found no difference in the result. The extracts were prepared with extreme care in order to avoid the possibility of bacterial contamination; the glands with the surrounding fat in which they are embedded were removed from the animal immediately after death and received in a recently boiled, corked or stoppered bottle; they were then taken to the laboratory and used at once, or the bottle was placed on ice until wanted. In either case the fat was removed from the glands with sterilised instruments and the gland immersed in 5 per cent. carbolic acid solution for 10 minutes; it was then washed in boiled salt solution and split into sectors with a sterile knife; the cortex was removed with sterilised curved scissors and the pieces washed in sterile salt solution and pounded with sterilised sand in a sterilised mortar. When the sand and pounded gland had formed a paste, a further quantity of sterile salt solution was added and the paste again treated by grinding and stirring. The brownish fluid obtained by this process was filtered into a sterile beaker through freshly boiled muslin, and the fluid so obtained was injected into the birds, care being taken to cleanse the skin at the site of injection and to seal the puncture, directly the needle was withdrawn, with collodion.

The usual site of injection was the loose tissue of the fold of the thigh, and although "knots," which we early found betoken necrosis of small masses of fat, regularly appeared within a day or two, the experiments were persevered in since they obviously produced no ill-effect on the general health of the bird or any painful local reaction. Usually the birds received an injection every second or third day. After a month or six weeks we found that the birds so injected not only failed to acquire the male plumage more rapidly, but were, in fact, tardy in assuming it. This applies especially to the young birds in their first plumage. From the similar experiments which we made on adult birds in "eclipse" it can equally be said that the injection produced no acceleration in the change of plumage. We do not consider that the retardation is to be ascribed to any specific effect of the injection, but regard it merely as an example of the general effect on plumage which any slightly unfavourable or unusual circumstance may produce. In the bird the intravenous method of injection is impracticable; and we found that very dilute solutions introduced subcutaneously produce the same necrosing effect as stronger ones. We did not try repeated intraperitoneal injections, for we considered that these could scarcely fail to produce such changes in general health as would tend to retard the normal plumage change. But although these experiments are vitiated by the necrosis produced, it is difficult to believe that none of the material introduced reached the circulation, and they may be tentatively taken to show that the cortex of the adrenal gland does not contain any body which has the specific effect of stimulating the appearance of secondary sexual characters.

We may turn now briefly to the local effects of cortical injection, although these are of small importance as compared with the original object we had in view.

The local effect of injection of a salt extract of the sheep's adrenal is very clearly shown by selecting the pectoral muscle for the site of experiment. A localised necrosis ensues, the necrotic tissue subsequently becoming encapsulated by new-formed granulation and fibrous tissue.

A healthy mallard received 1 c.c. of a salt extract of the cortex of a sheep's adrenals, deep in the substance of the left pectoral muscle, and another cubic centimetre into the loose fold of skin at the base of the right thigh.

The bird was killed seven days afterwards.

In the pectoral there was found a track of dry necrosed muscular tissue lying in a space about an inch in its longer diameter and bounded by a thin newly-formed fibrous capsule.

In the fold of the thigh there was, in the fat, a knot as large as a small haricot, which in section presented an opaque yellow centre and a translucent capsule.

A healthy mallard received into each pectoral muscle 1 c.c. of a salt extract of the cortex of a sheep's adrenals, the cortex used being carefully isolated from the medulla and twice washed in boiled salt solution.

The bird was killed nine days later. In each pectoral there was an encapsulated mass of dry necrotic muscle rather more than 1 cm. in length. In microscopic sections the dead tissue presents the typical appearance of a coagulation necrosis, the muscle fibres being hyaline and structureless. The sequestrum is closely surrounded by a zone of multinucleated giant cells, and beyond this there is newly-forming connective tissue consisting of elongated fibroblasts and intervening fibres, the latter being arranged in correspondence with the form of the necrosed focus.

A salt extract of the *medulla* of the sheep's adrenal likewise produces a local necrosis, on intramuscular injection.

A healthy mallard received into the pectoral muscle 0·5 c.c. of a salt extract of sheep's medulla. It was killed on the tenth day. In the substance of the muscle there was a dry firm sequestrum of muscular tissue surrounded by a fibrous capsule.

That the necrosis of the muscle, so resulting from the injection of medullary extract, need not be explained by a local spasm of the arterioles at the site of experiment appears from the fact that the cortical extract produces the same result. Cortical extract, as is well known from the original work of Oliver and Schäfer, has no immediate physiological effect upon the vascular system.

The necrosis must in both cases be attributed to the direct action of a toxic substance extracted from the cortex and from the medulla.

When similar injections of salt solution extract, whether of the cortex or of the medulla, are made into the tissues of the guinea-pig, œdema or suppuration not infrequently, though not invariably, follow. After injection of medullary extract we have seen extensive cavities containing serous or blood-stained fluid form amongst the muscles.

This result has been described also by Elliott and Tuckett* as following the introduction of pieces of sheep's adrenal, whether medulla or cortex, into the subcutaneous tissue of the same animal, viz., the guinea-pig.

These observers found that no such local results ensued if other animals than guinea-pigs were used to receive the grafts, and this, whether the adrenals grafted were taken from rats, guinea-pigs, or rabbits.

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* 'Journal of Physiology,' vol. 34, August, 1906.

They note that this inflammatory reaction at times failed to occur, and in this our results agree.

The conclusion at which the observers named arrive is that the subcutaneous tissues of the guinea-pig are peculiarly sensitive to adrenal grafts, which produce in them œdema and hæmorrhagic solution.

It is difficult, however, to eliminate here the possibility that the results are due to auto-bacterial infection.

The organs of normal guinea-pigs abound in various forms of bacteria, the growth of which is inhibited under natural conditions. When necrosis is set up by the adrenal graft or by the injection of an extract, the dead tissues furnish a nidus in which the latent pathogenic micro-organisms may grow.

Further Results of the Experimental Treatment of Trypanosomiasis: being a Progress Report to a Committee of the Royal Society.

By H. G. Plimmer, F.L.S., and H. R. Bateman, Captain R.A.M.C.

(Communicated by J. Rose Bradford, M.D., F.R.S. Received August 25, 1908.)

The following results are a continuation of the work of which summaries have already appeared in the 'Proceedings of the Royal Society.'*

The experiments have been carried out with the same strains of Nagana and Surra as were used before.

A.—*Condition of the Animals living at the Date of the Completion of the Tables in the last Paper.*

Table I.—Nagana Rats treated with Atoxyl and Succinimide of Mercury.

(Average duration of untreated disease, 5·5 days.)

No. 4 died on the 307th day after inoculation.
" 7 " 365th "
" 10 " 249th "
" 15 " 188th "
" 21 " 63rd "

Of these, No. 15, which was apparently cured, was used on the 147th day after inoculation for re-inoculation, with the view of ascertaining if any immunity had been conferred. This was found not to be the case.†

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* B, vol. 79, 1907, pp. 505—516, and B, vol. 80, 1908, pp. 1—12.

† *Vide* 'Roy. Soc. Proc.,' B, vol. 80, p. 10.

None of the above died with any of the signs of Nagana. Of the 21 rats tabulated,* only one died from trypanosomiasis, and this one was probably atoxyl-proof.

Table III.—Surra Rats treated with Atoxyl and Mercury Sozoiodol.

No. 5 died on the 286th day after inoculation from broncho-pneumonia.

Table VI.—Surra Rats treated with Atoxyl and Iodopin.

No. 9 died on the 221st day after inoculation from worms.

Rats treated with Atoxyl and Liq. Hydrarg. Perchlor.

The rat still living at the date of the last paper died on the 187th day after inoculation from broncho-pneumonia.

Rats treated with Sodium Antimonyl Tartrate. (*Table on pp.* 8—9 *of the last Paper.*)† *Results to August* 20.

No. 7 *is living and well* 354 *days after inoculation.*
„ 8 died on the 194th day „
„ 9 „ 323rd „ „
„ 10 „ 145th „ „
„ 14 „ 48th „ „
„ 15 „ 67th „ „
„ 16 „ 227th „ „
„ 17 „ 227th „ „
„ 19 „ 161st „ „
„ 20 „ 49th „ „
„ 21 „ 210th „ „
„ 23 „ 104th „ „
„ 25 „ 134th „ „
„ 26 „ 134th „ „
„ 27 „ 33rd „ „
„ 28 „ 34th „ „
„ 29 „ 57th „ „
„ 30 „ 28th „ „
„ 31 „ 147th „ „
„ 32 *is living and well* 346 *days after inoculation.*
„ 33 died on the 228th day „
„ 34 „ 188th „ „
„ 35 *is living and well* 346 *days after inoculation.*
„ 36 died on the 228th day after inoculation.
„ 38 „ 173rd „ „

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* 'Roy. Soc. Proc.,' B, vol. 79, p. 510.

† 'Roy. Soc. Proc.,' B, vol. 80.

It will be noticed that nine of the above rats lived for over 200 days, and nine others considerably over 100 days. Of those which have died only four have had recurrences (two had one, and two had three recurrences); none of them died with any symptoms of trypanosomiasis, and in none were trypanosomes found after death. An emulsion of the liver and of the bone-marrow of Nos. 9, 16, 17, 21, and 25 was injected into other rats with negative results in every case.

Rats treated with equal parts of 1 *per cent. Solutions of Sodium Antimonyl Tartrate and Sodium Arsenyl Tartrate.*

The five rats living at the date of the last paper have died:—

No. 1 died on the 199th day after inoculation.
„ 2 „ 26th „
„ 3 „ 43rd „
„ 4 „ 59th „
„ 5 „ 40th „

As was anticipated from the earlier experiments, this treatment has no advantages over that with antimony alone.

B.—*Further Experiments.*

Potassium Antimonate.

This was tried in doses up to 7 minims of a 1 per cent. solution. In this dose it was poisonous to rats, and it did not kill the trypanosomes.

Atoxyl and Sodium Antimonyl Tartrate.

This was given in doses of 5—7 minims of a 5 per cent. solution of atoxyl and of a 1 per cent. solution of sodium antimonyl tartrate. Three Nagana rats lived respectively 23, 43, and 41 days; they all had recurrences (the last had five) and died with living trypanosomes in the blood.

Pushing the drug does not have any good effect (see next table); the trypanosomes are not driven out more quickly, or more effectually; recurrences are more common, and inflammatory intestinal lesions were present in nearly every case. Two rats died with living trypanosomes in the blood: these two had become antimony-proof, as the later doses did not remove the trypanosomes from the blood, nor make any difference in their number. This strain did not maintain this quality for long, as after the second sub-inoculation from these rats the trypanosomes seemed to have become normal in their behaviour towards antimony. The strain was lost at this point.

Nagana Rats treated with Large Doses of Sodium Antimonyl Tartrate.

No.	Weight in grammes.	Total quantity of 1 per cent. sod. ant. tart. given, in c.c.	Recurrences.	Results.
1	200	5·75	1	Died on 30th day with intestinal lesions.
2	125	5·0	0	Died on 34th day with very marked intestinal lesions.
3	100	4·75	0	Died on 24th day: intestines necrotic.
4	100	4·0	0	Died on 108th day from pneumonia.
5	110	4·9	1	Died on 43rd day with inflamed intestines.
6	110	6·5	2	Died on 38th day with necrotic intestines.
7	175	8·5	2	Died on 40th day: trypanosomes in blood at death; paralysed.
8	110	9·0	2	Died on 45th day in similar condition to No. 7.
9	150	5·0	4	Died on 73rd day from gangrene of tail.
10	175	5·25	1	Died on 64th day from pneumonia.

Rats treated with Sodium Antimonyl Tartrate after Inoculation with Atoxyl-proof Trypanosomes.

The rats in the following table were all treated firstly with atoxyl to make sure that they were atoxyl-proof, and the treatment with antimony was then begun, when the trypanosomes were in large numbers in the blood.

	No.	Weight in grammes.	Total quantity of 1 per cent. sod. ant. tart. given, in c.c.	Recurrences.	Results.
Nagana...	1	100	3·0	1	Died on 29th day with trypanosomes in blood.
	2	100	1·0	0	Died on 11th day from nephritis.
	3	110	3·0	1	Died on 54th day.
	4	110	3·0	1	Died on 20th day with trypanosomes in blood.
	5	175	3·0	0	Died on 117th day from bronchopneumonia.
Surra......	6	100	3·0	0	Died on 27th day from septicæmia.
	7	145	5·75	1	Died on 41st day from pneumonia.
	8	140	4·0	1	Died on 38th day with trypanosomes in blood.

It will be seen that the atoxyl-proof strains of trypanosomes are less influenced by antimony than are the ordinary variety, as three of the above rats had living trypanosomes in the blood at death, and seven of them died at a very early date.

Rats treated with Antimony (Metal) and Sodium Antimonyl Tartrate suspended in a Fatty Medium.

Sodium antimonyl tartrate, when injected in watery solution into man produces very severe pain and inflammation in the neighbourhood of the injection, with more or less local necrosis. In order to make the use of antimony practicable in the form of injection, a series of experiments was undertaken, using various other media than water for solution or suspension of the antimony salt. Lanolin, olive oil, and sesamum oil were tried, but the results were not good. Finally, the medium Colonel Lambkin devised,* consisting of palmitin and antiseptics, which is used very largely for the intramuscular injection of calomel and mercury in syphilis, was tried, with the results which are set forth in the tables below.

One great advantage of these preparations is that they can be used upon man with far less difficulties and after-consequences than the watery solutions, which seem to be impracticable; this is of importance should antimony be found of use in human trypanosomiasis.

Major Ward, R.A.M.C., has used both the forms mentioned above on men for other purposes, and he has very kindly placed his notes at our disposal. In one of his cases four doses of ½ grain of sodium antimonyl tartrate, suspended in Colonel Lambkin's medium, were given intramuscularly into the buttock, the intervals between the doses being 3, 4, and 3 days: the doses were then increased to 1 grain, and seven doses of this strength were given, the intervals being 4, 3, 2, 4, 2, and 13 days. In all, this patient had 9 grains of the salt. Major Ward says that "the injections caused a certain amount of tenderness and discomfort at the seat of injection," but that is very different to the effects noticed after the injection of the watery solution.

Major Ward also treated two patients with antimony itself in a state of extremely fine division suspended in Colonel Lambkin's medium; they were each given one dose of 1 grain, and 11 days afterwards ½ grain. This form caused both pain and discomfort, and also a general increase in the size of the buttock, into which the injection was made, but this subsided without suppuration. This form would appear, however, to be much the more powerful of the two, as the effects obtained from the 1½ grains of the metal were as good and as lasting as those observed in the case in which 9 grains of sodium antimonyl tartrate were given.

The following tables show the results obtained in rats with sodium antimonyl tartrate and antimony prepared as mentioned above:—

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* 'Journ. Roy. Army Med. Corps,' 1906.

Sodium Antimonyl Tartrate, 5 *per cent., in Colonel Lambkin's Medium.*

	No.	Weight, in grammes.	No. of doses given.	Total quantity given, in minims.	Recurrences.	Results to August 20.
Nagana.	1	100	4	18	2	Died on 53rd day: bad itch.
	2	100	2	6	1	Died on 16th day with intestinal lesions.
	3	115	1	2	0	Died on 113th day of pneumonia.
	4	150	1	2	0	Died on 19th day of pneumonia.
	5	110	5	17	1	Died on 27th day: abscess.
	6	125	3	13	0	Died on 26th day of pneumonia.
	7	150	3	13	0	Died on 89th day: itch.
	8	175	1	3	1	Died on 24th day: abscess.
Surra.	9	200	3	17	1	Died on 40th day of injury.
	10	225	3	13	2	Died on 28th day with intestinal lesions.
	11	115	2	6	1	Died on 22nd day of pneumonia.
	12	220	11	33	7	Died on 66th day with acute intestinal lesions.
	13	125	1	3	0	*Living and well* 154 *days after inoculation.*

None of the above have died from trypanosomiasis. In rats the intramuscular injection of even a few minims is difficult, and if any of the material be left under the skin a slough forms, which takes a long time to heal. It will be noticed that No. 3 had only one dose: the rat lived 113 days and died of pneumonia; inoculations made from its organs were negative. No. 13, which is still living (154 days), has also had only one dose.

From the following table it will be seen that the administration of the metal itself has a considerable effect on the trypanosomes: it has a distinctly better effect on Surra than upon Nagana, four Surra rats out of 16 being still alive, and four others having lived for a long time. In none of the Surra rats were trypanosomes found at death, whereas in three of the Nagana rats they were present. The metal is much more irritating than the tartrate, but the effect is in most cases more prolonged; this is probably due to the fact that the absorption of the metal is much slower. Further, the smaller doses would appear to be the most efficient.

Antimony, in a state of very fine division, 5 *per cent., in Colonel Lambkin's Medium.*

	No.	Weight, in grammes.	No. of doses given.	Total quantity given, in minims.	Recurrences.	Results to August 20.
Nagana.	1	100	3	12	3	Died on 61st day: abscess.
	2	200	3	11	2	Died on 29th day with trypanosomes in blood.
	3	100	3	9	1	Died on 34th day with intestinal lesions.
	4	110	3	12	1	Died on 15th day with trypanosomes in blood.
	5	100	4	14	1	Died on 27th day with intestinal lesions.
	6	150	4	15	2	Died on 26th day with intestinal lesions.
	7	150	6	23	3	Died on 33rd day with trypanosomes in blood.
	8	300	3	13	4	Died on 51st day from broncho-pneumonia.
	9	300	2	8	2	Died on 36th day from broncho-pneumonia.
	10	200	2	8	0	*Living and well* 204 *days after inoculation.*
	11	300	2	8	1	Died on 29th day: abscess.
	12	250	2	8	1	Died on 39th day from broncho-pneumonia.
	13	250	2	8	7	Died on 92nd day from pneumonia.
	14	300	1	5	0	Died on 32nd day: fatty liver.
	15	300	1	5	0	Died on 30th day.
	16	150	1	3	0	Died on 74th day from pneumonia.
Surra.	17	110	1	5	0	*Living and well* 230 *days after inoculation.*
	18	125	4	17	4	Died on 146th day: one recurrence took place after an interval of 12 weeks.
	19	250	4	35	3	Died on 48th day from broncho-pneumonia.
	20	225	1	4	0	Died on 39th day from broncho-pneumonia.
	21	100	1	4	0	Died on 44th day: itch.
	22	100	3	10	1	Died on 44th day: itch.
	23	150	2	9	1	Died on 216th day of pneumonia.
	24	100	3	12	3	Died on 94th day: abscess. One recurrence took place after an interval of 46 days.
	25	100	2	9	0	*Living and well* 253 *days after inoculation.*
	26	150	2	9	0	Died on 29th day from pneumonia.
	27	125	1	3	0	*Living and well* 226 *days after inoculation.*
	28	100	1	3	0	Died on 91st day: itch.
	29	100	1	3	0	*Living and well* 226 *days after inoculation.*
	30	125	1	4	0	Died on 27th day from pneumonia.
	31	100	1	4	0	Died on 27th day from pneumonia.
	32	150	1	4	0	Died on 41st day: itch.

Experiments in which Antimony and Sodium Antimonyl Tartrate were given before Inoculation, in order to Test their Effects upon the Development of the Disease.

In these experiments the substances were given suspended in Colonel Lambkin's medium, and it will be noticed that the metal is far more effective in delaying the appearance of the trypanosomes in the blood than is the salt: this is probably due to the slower elimination of the metal. This method, if the doses were repeated, might be of some practical value in getting animals safely across dangerous tracts of country.

Four Rats were given 5 minims of 5 per cent. Antimony (metal) Cream on December 19. In Nagana the trypanosomes usually appear in the blood on the second or third day after inoculation.

No.	Inoculated with Nagana on—	Trypanosomes appeared in the blood on—
1	December 20……	December 31, the 11th day.
2	„ 21……	January 2, the 10th day.
3	„ 23……	December 31, the 8th day.
4	„ 24……	„ 30, the 6th day.

Four Rats were given 3 minims of 5 per cent. Sodium Antimonyl Tartrate Cream on December 19.

No.	Inoculated with Nagana on—	Trypanosomes appeared in the blood on—
1	December 20……	December 25, the 5th day.
2	„ 21……	„ 24, the 3rd day.
3	„ 23……	„ 26, the 3rd day.
4	„ 24……	„ 27, the 3rd day.

Rats treated with Lithium Antimonyl Tartrate.

There are differences in the effects produced by the potassium, sodium, and lithium antimonyl tartrates, if given under similar conditions and dosage. The commercial potassium salt is very impure. The pure sodium and lithium salts which we have used have been prepared for us by Dr. R. H. Aders Plimmer, of University College. The sodium salt contains roughly about 2 per cent. more antimony than the potassium salt, and the lithium salt contains about 2 per cent. more antimony than the sodium; but the doses of the lithium salt have to be much smaller than the corresponding doses of the sodium salt. For instance, 0·5 c.c. of a 1 per cent. solution of the lithium salt is fatal to a rat of 125 grammes, and 0·39 c.c. is fatal to a rat of 80 grammes. When the watery solution is injected intramuscularly, it has not caused necrosis of the tissues in rats, but subcutaneously it has occasionally done so. We have found that the best strength of solution for rats is 0·25 per cent., and of this 0·5 c.c. has been given for a dose.

The following table shows the effects of this dosage.

	No.	Total quantity in c.c. given of 0·25 per cent. solution.	Recurrences.	Results to August 20.
Nagana...	1	7	2	Died of disease on 47th day.
	2	7	1	Killed on 47th day, owing to abscess. Emulsion of organs injected into another rat gave negative result.
	3	4·5	1	Allowed to die on 34th day, the date of recurrence.
	4	4·5	0	*Alive and well* 134 *days after inoculation.*
	5	4	0	*Alive and well* 125 *days after inoculation.*
Surra......	6	4	0	*Alive and well* 134 *days after inoculation.*
	7	4	0	Died on 42nd day of broncho-pneumonia: no evidence of trypanosomes.
	8	4	0	*Alive and well* 134 *days after inoculation.*
	9	5	2	Died of disease on 47th day.
	10	4·5	0	*Alive and well* 125 *days after inoculation.*
	11	3·5	2	Died of disease on 18th day: itch.
	12	3	1	Allowed to die: relapse not treated.

From the above it will be seen that five out of these 12 rats are alive and well at periods varying from 125 to 134 days. This salt is much more soluble than either the potassium or sodium compound, which may, perhaps, as well as its greater antimony content, account for its greater effectiveness. It is, however, more irritating subcutaneously in watery solution.

Experiments with Antimony upon Dogs.

In order to see what the effects of antimony would be on the larger and more important animals when suffering from trypanosomiasis, a series of experiments on dogs has been begun. The trypanosome used was that of Surra, which kills dogs of about 20 lbs. in weight in approximately 14 days, as this is the trypanosome which is of practical importance with regard to dogs.

Dr. MacConkey, of the Lister Institute, kindly performed some initial experiments for us. He made some experiments with a 20 per cent.

suspension of sodium antimony tartrate in Col. Lambkin's medium, in order to find the minimum lethal dose, and he found that 0·5 c.c. of this 20 per cent. cream per 10 lbs. of body weight was probably about the full dose. But he also found that, for practical purposes, this 20 per cent. cream was much too strong, as it caused sloughing in every case; indeed, dogs do not seem to bear these suspensions as well as they do solutions. Dr. MacConkey kept one dog alive till the 40th day, and one until the 30th, but both had many relapses.

Profiting by the experience thus gained, we have made further experiments using much smaller doses, with satisfactory results. But we have found, since trying the lithium antimonyl tartrate that this acts more effectually and with less irritation than the creams, whether of metal or salt. All the animals tabulated below are in good condition and are gaining in weight.

(Average length of untreated disease, 14 days.)

No.	Weight, in lbs.	Total quantities given, in minims.	Recurrences.	Results to August 20.
1	22	Antimony cream, 5 per cent., 60 m. ...... Sod. ant. tart., 2 per cent., 40 m.	2	No trypanosomes present since July 13; is alive and well on the 62nd day after inoculation.
2	22	Sod. ant. tart., 2 per cent., 80 m. .... Lith. ant. tart., 2 per cent., 55 m.	2	Had 9 pups during treatment; there were 42 days between the two recurrences; is alive and well on the 62nd day after inoculation.
3	37½	Sod. ant. tart. cream, 5 per cent., 80 m. Sod. ant. tart., 2 per cent., 40 m. Lith. ant. tart., 2 per cent., 90 m.	6	Incorrect dosage seems a probable cause of these relapses; is alive and well on the 62nd day after inoculation.
4	16	Sod. ant. tart. cream, 5 per cent., 40 m. Sod. ant. tart., 2 per cent., 20 m. Lith. ant. tart., 2 per cent., 40 m.	3	Is alive and well on the 53rd day after inoculation.
5	13½	Sod. ant. tart. cream, 5 per cent., 35 m. Lith. ant. tart., 2 per cent., 47 m.	3	Is alive and well on the 53rd day after inoculation.

The second and third substances mentioned in Column 3 were given at the recurrences. That these dogs are all alive and well encourages us to hope that, as we get a better knowledge of the dosage required, the recurrences may be less frequent.

Experiments made with Rats treated with Antimony, in order to find out in what Organs the Trypanosomes are latent.

The following initial experiments were made in order to find out where the trypanosomes rest during the period in which the peripheral blood is free from them, after treatment with antimony. Further experiments are being carried on for the purpose of ascertaining in what forms they are present in the organs of treated animals.

The rats were inoculated with Nagana, which is less affected by antimony than Surra, and were all treated with four doses of sodium antimony tartrate. The rats were killed at various intervals, and the organs selected (the liver and bone-marrow) were made into an emulsion with a minimum quantity of 0·75 per cent. salt-solution, and injected into other rats in doses of 1 c.c.; the same dose of blood from the heart was also given.

In the following table the signs + and — are used respectively to denote a positive or negative result.

No.	Number of days after treatment upon which rats were killed.	Blood.	Liver.	Marrow.
1	7	—	—	—
2	10	—	—	—
3	12	—	—	—
4	14	—	—	—
5	2	—	+	+
6	4	—	+	—
7	3	—	+	+
8	12	—	—	+
9	14	—	—	+
10	16	—	—	+
11	20	—	—	+

From this table it would appear that the bone-marrow is the place where the trypanosomes can live longest, and that the liver is also a place where they can find protection. This is borne out by some experiments we have made upon trypanosomiasis in birds, in which cultivations of trypanosomes can often be made from the bone-marrow when they cannot be made either from the organs or the blood. The doses given to the above rats were rather under those which we should judge to be curative, but in four cases the results were entirely negative.

Complete Survey of the Cell Lamination of the Cerebral Cortex of the Lemur.[*]

By Dr. F. W. MOTT, M.D., F.R.S., and AGNES M. KELLEY.

(Received March 6,—Read April 2, 1908.)

(From the Pathological Laboratory of the London County Asylums, Claybury.)

[PLATES 14—18.]

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I. NOTES ON MATERIAL AND METHOD.

It has been endeavoured by a microscopical examination of series of sections of the cerebral cortex of the lemur to map out the extent and boundaries of the types of cell lamination observed. The brains of four lemurs were used:—One *Lemur brunneus,* one *Lemur mongoz,* and two specimens of *Lemur catta.*

The tissue was divided into small blocks about 3 inches thick, cut as far as possible at right angles to the main fissures. Two hemispheres were cut up completely, each into about 24 blocks, and the others were used to supplement these. To ensure greater accuracy in the results, the blocks were cut out in varying positions from the different hemispheres, so that any discrepancy in one set of sections might be checked in another. The positions from which these blocks were taken were mapped out on drawings of the surface of the brain, both the blocks and the key being numbered.

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[*] A Government Grant of £20 was made to defray the expenses of this research.

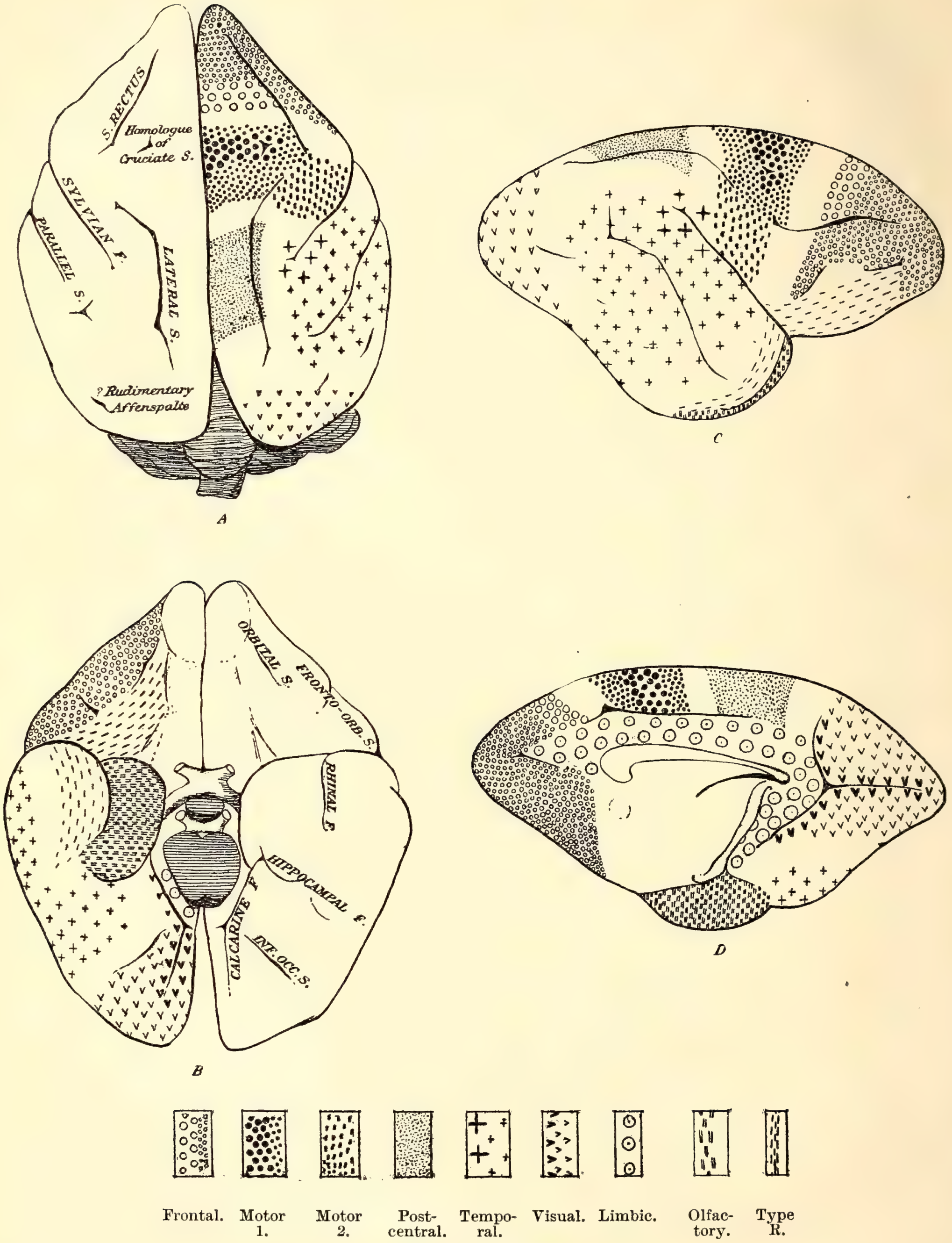
The specimens in the first place were hardened in formalin. The small blocks were passed through several changes of alcohol of gradually increased strength, dehydrated in absolute, cleared in xylol, and imbedded in paraffin. Sections $10\,\mu$ in thickness were cut in series and numbered, and about one section in every 20 was stained, the Nissl or the polychrome methylene blue method being used.

To map out the various areas, their extents were first marked on the plans of the sections (*vide* figs. 3 and 4). By applying these plans to the diagrams of the surface of the brain, fig. 1 has been composed.

The drawings of the types of cortical lamination (Plates 14—18) were made with the camera lucida, at a magnification of 120 diameters (figs. 5 to 14).

II. A SHORT ACCOUNT OF THE LEMUR AND CORRELATION OF ITS MODE OF LIFE AND HABITS WITH THE CORTICAL DEVELOPMENT OF THE BRAIN.

The true lemurs are found only in Madagascar, and frequent the forest in large numbers. Small, agile animals, hardly the size of a cat, with fox-like faces, large ears, long tails, and thick fur, they are eminently adapted to an arboreal life. They are able to run along the branches with great rapidity, using all four limbs, but they also spring from branch to branch with ease. The thumb and great toe are opposable, a feature which is not found in lower mammals, but which is characteristic in the primates. At the same time they seem to prefer to seize their food with their mouths, and do not grasp with the fore limb so readily as the apes. Though active and playful and fairly easily tamed, these animals, as compared with the monkeys and apes, are not very intelligent. Their food consists of fruit, nuts, and also insects and birds' eggs. They are nocturnal or diurnal, and are probably guided in their search for food to a large extent by their sense of smell. The olfactory region of the brain, with the olfactory tracts and bulbs, is extremely well developed, and very much larger and more extensive proportionately than that of the primates. The large ears and well-developed semicircular canals may be correlated with the comparatively large temporal lobe and an acuteness of hearing obviously useful to a nocturnal feeding animal. The eyes, which are large, are set wide apart, so that the visual axes cannot be parallel; consequently, the animal does not, like the ape, possess convergent binocular stereoscopic vision. This fact finds anatomical expression in lack of macula, relatively smaller optic nerves, and absence in the brain of a definite occipital lobe.


S. RECTUS
Homologue of Cruciate S.
SYLVIAN F.
PARALLEL S.
LATERAL S.
? Rudimentary Affenspalte
A
C
ORBITAL S.
FRONTO-ORB. S.
RHINAL F.
HIPPOCAMPAL F.
CALCARINE F.
INF. OCC. S.
B
D
Frontal.
Motor 1.
Motor 2.
Post-central.
Temporal.
Visual.
Limbic.
Olfactory.
Type R.

Key to the Areas.

Fig. 1.—*Lemur mongoz.*

A. Diagram of upper surface of brain. C. Lateral view of right hemisphere.
B. " under " D. Mesial surface of right hemisphere.

In B, C, and D the cerebellum has been removed. The various markings on the right hemisphere indicate various cortical areas, as shown by the key.

III. GENERAL DESCRIPTION OF THE BRAIN.

The lemur's brain measures about 5 cm. by 3·5 cm., and weighs on an average about 19 grammes. The surface is broken by few fissures. There is no occipital lobe, the cerebellum being only partially covered by the cerebral hemispheres (*vide* fig. 1).

The olfactory bulbs and tracts are comparatively large, and the rhinal fissure is seen on the lateral surface of the temporal lobe. It is therefore evident that the lemur has a well-developed olfactory area.

On the dorso-lateral surface there are four main fissures, which, according to the nomenclature of Elliot Smith (1), may be termed the sulcus rectus, the lateral sulcus, the Sylvian fissure, and the parallel sulcus. Two small fissures are found on the fronto-orbital surface—the orbital, near the anterior pole, and behind it the fronto-orbital. On the mesial surface are seen the bifurcated calcarine fissure, the intercalary sulcus, and the hippocampal fissure. The latter gives off a branch in front of a small hippocampal tubercle. A short fissure, called by Elliot Smith the inferior occipital sulcus, lies on the under surface of the occipital region. In some species a small sulcus rostralis can be seen on the fronto-mesial surface. To these more prominent fissures may be added a few others, which though small and shallow are constant. The most anterior of these lies in the middle of the triangular space formed on the dorsal surface by the sulcus rectus, the anterior part of the sulcus lateralis, and the border of the mesial surface, and is probably the homologue of the sulcus cruciatus of the carnivora. Between the superior extremities of the Sylvian fissure and of the parallel sulcus a small series of fissurets is usually present, tending to join the two sulci. This has been pointed out by Elliot Smith, who observes that in the aye-aye the communication is complete, as in the lower mammals, and the two fissures form one continuous arch; but that in the primates these fissures are always separated. Some shallow linear depressions are seen near the margin of the posterior pole. The superior of these, which is constant although it varies a little in position, is possibly an indication of a rudimentary "Affenspalte."

The lemur's brain forms an interesting study for comparison with the brains of carnivora, ungulates and the apes. Whereas in size, in simplicity of convolutional pattern, in the uncovered cerebellum, and in the well-developed olfactory area and bulbs, it is allied to the lower mammals', in many respects it bears resemblance to the ape's brain; notably in the bifurcated calcarine fissure, the separate intercalary sulcus, the more completely developed Sylvian fissure, and the separation of this fissure from the parallel sulcus.

In comparing a lemur's brain with an ape's, the difficulty of distinguishing the homologue of the Rolandic fissure presents itself. It has been suggested that the small dimple in front of the sulcus lateralis is the possible homologue of Rolando; but both stimulation experiments and histological examination show that this fissure is *within* the motor area (*vide* figs. 1 and 2). It therefore does not fulfil the condition of forming the posterior boundary of the motor area which is characteristic of the fissure of Rolando in the apes and higher anthropoidea. It seems more probable that it is the homologue of the cruciate sulcus of the carnivora. Campbell has pointed out that such a homologue exists within the motor area in the ape and in man, and finds it in the paracentral lobule. He further observes that: "Just as in the lower animals, the deposit of giant cells clings to the sulcus cruciatus, so it is with this fissuret; in mapping

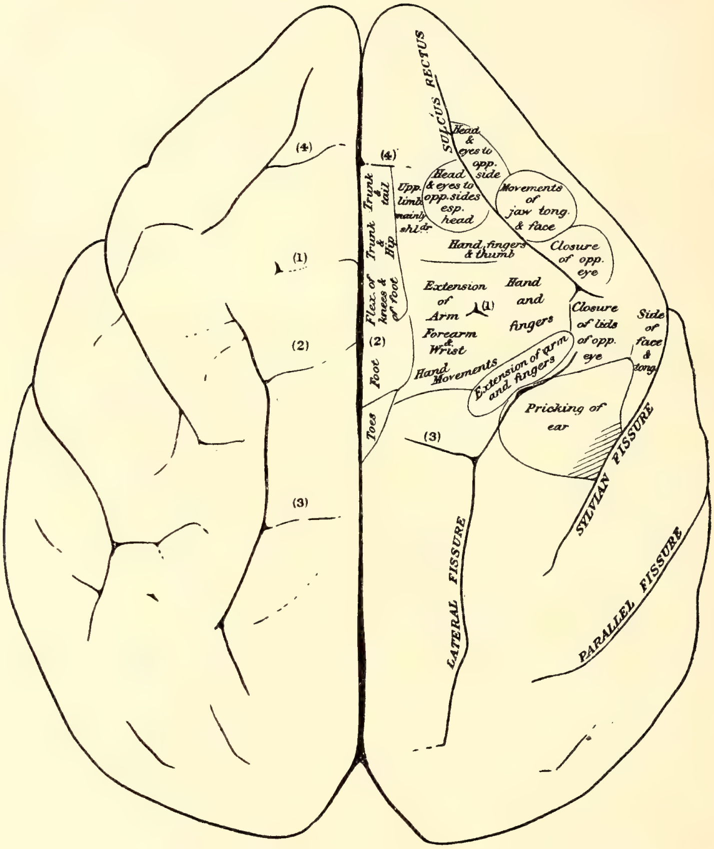

(4)
(4)
(1)
(2)
(3)
(1)
(2)
(3)
SULCUS RECTUS
Head & eyes to opp. side
Head & eyes to opp. sides esp. head
Movements of jaw tong. & face
Upp. limb mainly shl.dr
Trunk & tail
Trunk & Hip
Flex. of knees & of foot
Hand, fingers & thumb
Closure of opp. eye
Extension of Arm
Hand and fingers
Forearm & Wrist
Closure of lids of opp. eye
Side of face & tong.
Foot
Hand Movements
Extension of arm and fingers
Toes
Pricking of ear
SYLVIAN FISSURE
LATERAL FISSURE
PARALLEL FISSURE

Fig. 2.—Diagram of Lemur's cerebrum, seen from above. The motor area, as ascertained by the stimulation method, is shown on the right hemisphere.

out the distribution of the motor area, I have, in all, examined the paracentral lobule in serial sections quite a dozen times in man and three times in the anthropoid ape, and I have invariably detected clusters of giant cells in the walls of the fissuret, not all along, perhaps, but always in its upper part." It is interesting to note that in the cerebral cortex of the lemur the largest Betz cells were found around this fissuret. As the anterior portion of the lateral sulcus is the posterior boundary of the motor area for about 5 mm., it seems possible that that part of the fissure may be the homologue of Rolando, or of one of its elements; but, except at this point, it is

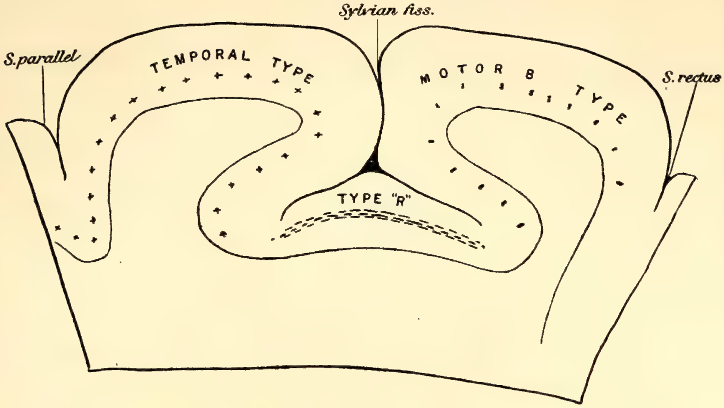

Sylvian fiss.
S.parallel
TEMPORAL TYPE
MOTOR B TYPE
S. rectus
TYPE "R"

Fig. 3.—Diagram of section cut at right angles to Sylvian fissure about half-way between its upper extremity and the rhinal fissure, showing the submerged area of cortex which lies within its operculated walls.

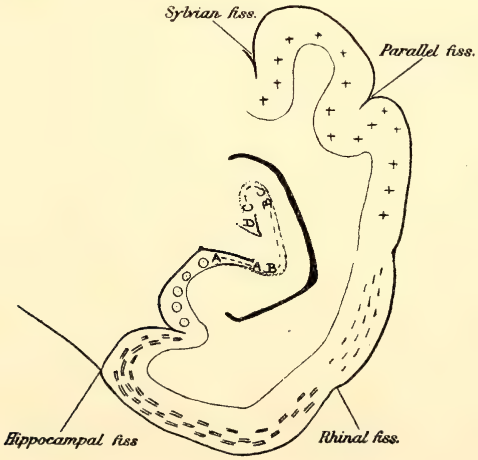

Sylvian fiss.
Parallel fiss.
C
B
C
D
A
A.B.
Hippocampal fiss
Rhinal fiss.

Fig. 4.—Section through right temporal lobe of Lemur's brain.

noticeable that just as in the occipital region there is no definite *Affenspalte* present forming a boundary to the visual area, so in the central region there is no fully formed fissure making a sharp line of demarcation between the motor and post-central areas.

IV. Histological Description of the Cortex.

Summary of Histological Description of the Cortex.

By comparing the camera lucida drawings of typical strips of cortex of the frontal, motor, post-central, visual, temporal, and hippocampal areas (*vide* Plates 14—18),* it will be seen that although the differences in the types are not so pronounced as in the higher apes and man, it is evident that each of these areas has a characteristic lamination. The cortex of the *archipallium* being peculiar both in its lamination and in its cell elements, is easily mapped out. The *motor* area is characterised by the Betz cells; the *frontal* area by a comparative poverty of cells, both in size and number; and neither the frontal nor the upper part of the motor area possesses a definite layer of granules. The *sensory areas*, on the other hand, are distinguished by a rich granule layer; and of these the *visual* area is marked by the large solitary cells of Meynert and the line of Gennari; the post-central area by a line of large pyramids below the granules; and the temporal area shows a line of peculiar elongated pyramids which are especially large and conspicuous in the region about the posterior end of the Sylvian fissure. A detailed description of the histology of these areas is given below.

These typical forms of lamination are found in regions which have been termed "focal." They are generally surrounded by a cortex less typical or "peri-focal." Between some types (for example, the motor and post-central) the peri-focal area of the one merges into the peri-focal area of the other, with the result that there is a region between the two which shows mixed characteristics. We have found that, in the lemur's cortex, there are few definite border lines where one type of cortex ends abruptly and another begins. On microscopical examination of the Rolandic region of the ape's cortex the change from the motor to the post-central type can be readily observed (the base of the fissure of Rolando forming the approximate boundary). In the lemur the motor cortex merges very gradually into the post-central type. This is the case with most of the areas within the neopallium. There is, however, a fairly definite boundary line between the neopallium and the archipallium. In making the diagrams we have, therefore, not attempted to map out the whole cortex into arbitrary divisions. We have marked the "focal" areas in each case with larger figures, and the "peri-focal" areas with figures of the same type but smaller. Blank spaces have been left on the diagrams to indicate the intermediate indefinite areas which show mixed characteristics, as it cannot be definitely stated that they belong to the one type or to the other.

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* Plates 14—18, containing figs. 5—14, represent the cell lamination of the cortex in typical areas of the lemur's brain. The drawings were made with the camera lucida, the magnification being 120 diameters. A description of these areas is given in the text.

Special mention may be made of the intermediate areas which lie anteriorly to the visual area, both on the mesial and lateral surfaces. The cortex in this region of the brain is particularly rich in cells. The pyramidal layer is well developed, the individual cells being rather smaller and more numerous than the pyramids of the post-central and temporal types, but not so small and less closely packed than those of the visual area. The granule layer forms a conspicuous band, rather deeper though again less closely packed than in the visual area, and the individual cells here also are rather larger. A line of darkly staining stellate cells is seen above the granule layer, and a line of large pyramids is found which resemble those seen in the temporal area but which are not so large as those found in its most typical, or "focal" region.* We have not mapped out this cortex as a definite type covering a definite area. Though at its posterior border the point at which it ends and the visual type begins can be observed in longitudinal sections of this part of the brain, its anterior border is very indefinite. It merges into the temporal and post-central types so gradually that unless sections from the focal regions of these areas are compared with a section from its posterior margin, no very appreciable differences in structure can be seen. The cortex of this region thus seems to be intermediate in structure—as it is in position—to the post-central, temporal, and visual types. It is probable that in this area lie the homologues of the "parietal" and "visuo-psychic" types described by Campbell in the higher apes. These types would tend to become more definite in character and extent as the parietal lobe becomes more highly developed.

Motor Area.†

Extent and Boundaries: Motor A ▨.—This type covers that part of the dorsal and mesial surfaces which lies between the posterior end of the sulcus rectus, the anterior end of the sulcus lateralis, and the intercalary sulcus (*vide* fig. 1). Posteriorly and anteriorly this type becomes less characteristic, and gradually merges, in front, into an area intermediate to the motor and the frontal types; and behind, into one intermediate to the motor and the precentral type. This lamination is continued further forward within the sulcus rectus than can be indicated on the surface diagram.

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* It is regretted that, owing to the number of illustrations being necessarily limited, a reproduction of the drawing of this type cannot be given.

† This area was mapped out by the experimental method by Professor Halliburton and Dr. F. W. Mott, and has been described in a paper now in press for the 'Proceedings of the Royal Society.'

Motor B ▨.—Type "Motor B" covers the space which lies between the extremities of the sulcus rectus and the sulcus lateralis, and extending downwards on to the superior wall of the Sylvian fissure is bounded inferiorly by the sulcus which lies at its base. (A more particular description of this sulcus is given under "Type R.") Superiorly it merges into "Motor A," anteriorly into an area intermediate to this type and type "Frontal B," and posteriorly into one intermediate to this type and the temporal and post-central types.

Characteristics: Motor A. The cortex is about 2 mm. deep—the molecular layer measuring about 0·17 mm., the pyramidal layer and granules together about 0·9 mm., the pallid zone in which the Betz cells lie about 0·2 mm., and the polymorph layer about 0·7 mm. (*vide* fig. 5, Plate 14). The cells of the pyramidal layer are larger and have more processes than in other parts of the cortex (those of the post-central and temporal types most nearly approaching them in form). They are somewhat irregularly arranged, owing perhaps to the presence of the fibres from the Betz cells. Granules are scattered in fair numbers at the bottom of the pyramidal layer; but they do not form a distinct layer. The infra-granular pyramids are the most typical features of this area. They are for the most part well-formed Betz cells, containing Nissl bodies and having several branched processes. They frequently measure as much as 60 μ by 25 μ, and are sometimes larger. This line of cells seems to occupy a pallid zone in which only a few other cells are scattered—these being smaller Betz-like cells, faintly-stained pyramidal cells, and a few granules. Some of the large Betz cells closely resemble the typical giant Betz cells of the cortex of the higher apes, but many are more pyramidal in shape. The tendency to arrangement in nests which has been described in the human cortex is not general, though it can sometimes be seen. The largest Betz cells are found immediately before and behind the small fissuret which lies in the middle of this area, and between it and the intercalary sulcus.

Motor B (*vide* fig. 6).—In "Motor B" the pyramidal cells are smaller than in "Motor A"; a line of darkly staining stellate cells is scattered above or among the granules, and the granules form a fairly well-marked line.

The cells which correspond in position to the Betz cells in "Motor A" are not conspicuous either in size, staining, or number. But a somewhat scattered line of cells can be seen measuring about 25 μ by 15 μ which are Betz-like in shape, having many branched processes and Nissl granules. From the facts that the movements of the head and eyes, mouth, closure of opposite eye, side of face and tongue, and pricking of ears have been obtained by stimulation of this area, and that the cells here described are present, it may be inferred that this area is motor in function, although it differs considerably from the typical motor cortex in the characteristics mentioned above. The presence of stellate cells and granules points to a sensory type. This area is probably a sensori-motor area which is experimentally excitable, giving rise to definite movements; and corresponding to another definite sensori-motor area—the calcarine visual cortex, which, in the ape, gives rise to definite movements on stimulation. But for reasons which have been given in Addendum (p. 505), the visual cortex has not been experimentally proved to be excitable in the lemur.

Frontal Area.

Extent and Boundaries: Frontal A ▨.—This area forms a band about 1 mm. in width across the dorso-mesial surface from the sulcus rectus to the intercalary sulcus. It is bounded anteriorly by "Frontal B"; posteriorly it extends nearly to the level of the end of the sulcus rectus, and there merges into an intermediate area which lies between this type and the motor area.

Frontal B.—Type "Frontal B" covers the anterior pole of the hemisphere, and extends: Posteriorly, on to the dorsal and mesial surfaces for about 2 mm. backwards, being bounded by "Frontal A;" on the mesial surface to the intercalary sulcus, and, further forward, to an imaginary line continuous with this sulcus to the pole; inferiorly, to the orbital and fronto-orbital sulci.*

Characteristics: Frontal A.—The cortex is about 2 mm. deep, the molecular layer measuring about 0·2 mm., the pyramidal layer about 1 mm., and the polymorph layer about 0·7 mm. (*vide* fig. 7, Plate 15). The pyramidal cells are not closely packed, and incline to a longitudinal columnar arrangement. There are no really large pyramidal cells either above or below the granules, the largest measuring about 14 μ by 7 μ. A number of granules are scattered at the bottom of the pyramidal layer, but they hardly form a distinct layer.

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* A comparison with the brains of the primates, described by Campbell, suggests that an "orbital" type of cortex would be found in this position. We have not, however, found sufficient histological evidence to justify mapping out a separate type here, although it may be noted that in sections of so small a brain the cortical lamination of this region is often distorted, owing to the sections being necessarily cut through the walls of the short, branched orbital sulcus. It is thus difficult to make exact observations on this part of the cortex.

Frontal B.—This area is very poor in cells. Those of the pyramidal layer are more scanty than in other parts of the neopallium; the largest cells are still smaller than in "Frontal A," and the granules are still less prominent.

Sensory Areas.

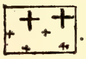
Whereas the archipallium and the frontal and motor areas (except the cortex described under "Motor B") have no definite line of granules, the cortex covering the posterior half of the hemisphere and the temporal lobe is characterised by a deep granule layer, which indicates a sensory type of cortex.

This conspicuous layer of granules is seen throughout the whole area, but there are various other distinctions in different parts of it, and it has been subdivided accordingly into the temporal type, the post-central type, and the visual type. Although distinct variations seem to justify these subdivisions it must be pointed out that the changes from one type to another in this region are gradual, and that therefore the boundaries can only be approximately given. Stellate cells are seen among the granules, but they are more numerous and more typical in the temporal and visual regions than in the post-central.

Post-central Area ▨.

Extent and Boundaries.—The post-central type of cortex lies on the dorsal and mesial surfaces, between the motor and visual areas. Anteriorly, about 6 mm. behind the anterior extremity of the lateral sulcus it merges into an area intermediate to it and the motor area; and, posteriorly, near the posterior end of the lateral sulcus into one intermediate to it and the visual area. Inferiorly, on the dorsal surface, it extends across the lateral sulcus, where it merges into the temporal type, and on the mesial surface it extends to the limbic cortex. The change from the post-central to the temporal types is so gradual as to be scarcely perceptible, and it is only by comparing the upper part of the post-central with the temporal area that the differences in type become apparent.

Characteristics.—The cortex is about 1·8 mm. in depth; the molecular layer measuring about 0·15 mm., the pyramidal layer about 0·8 mm., the granules 0·2 mm., the polymorph layer about 0·6 mm. in depth (*vide* fig. 8). The pyramidal cells are rather more numerous than in the motor area (A), and are not so large, and are more regularly arranged. At the bottom of the layer is a line of supra-granular pyramids which average about 7 μ by 2 μ in size. The granule layer is well marked, and above it and among the granules are scattered a few stellate cells. They are not, however, so numerous as in the temporal and visual areas. The line of large infra-granular pyramidal cells is characteristic of this area. They are plump pyramidal-shaped cells, measuring on an average 35 μ by 15 μ, having several branched processes and Nissl granules. These cells seem to correspond to similar cells described by Campbell as occurring in the post-central convolution in the primates.

Temporal Area .

Extent and Boundaries.—The "temporal type" covers the greater part of the temporal lobe. Inferiorly it extends to within about 4 mm. of the rhinal fissure, and there merges into type "R." Anteriorly it extends on to the inferior wall of the Sylvian fissure and ends abruptly at the gyrus which lies at its base; the lamination on the other side of the sulcus being type "R" (*vide* fig. 3). Behind this gyrus the temporal type crosses the base of the Sylvian fissure, and gradually merges into the post-central type. Curving round the posterior extremities of the Sylvian fissure and the parallel sulcus, and covering the rudimentary sulci behind the latter, it again merges gradually into the cortex which is intermediate to it and the post-central and visual types respectively. On the under surface the inferior occipital sulcus forms a boundary posteriorly, and more anteriorly it merges into an area which seems to be intermediate to it and the visual type.

Characteristics.—The depth of the cortex is about 2 mm., the molecular layer measuring about 0·15 mm., the pyramidal 0·7 mm., the granules 0·25 mm., the zone in which the large infra-granular pyramids lie about 0·2 mm., and the polymorph layer about 0·7 mm. in depth (fig. 9). The superficial cells of the pyramidal layer are small and rather closely crowded together; and among the more usual triangular cells may be seen a few which are quadrilateral in shape. The remainder of the pyramidal layer is formed of well developed pyramids, which are larger and less closely packed than those of the visual area, and more regularly arranged than in the motor. The largest cells at the bottom of the layer measure about 30 μ by 7 μ. The granule layer is conspicuous. The individual cells are larger and less crowded together than in the visual area. A few small pyramids are mingled with them. Scattered among the granules, or immediately above them, is a line of darkly staining stellate cells, of irregular angular shapes, giving off many processes. They measure on an average 25 μ by 18 μ, and are more conspicuous in shape and staining than in size. A line of large infra-granular pyramids is very characteristic of this region. These are darkly staining, thin, elongated pyramids, measuring from 25 μ by 9 μ to about 50 μ by 10 μ.

This lamination is found in its most typical form on and around the upper portion of the inferior wall of the Sylvian fissure, which area has, therefore, been termed "Temporal A." As it crosses the base of the fissure and ascends on to the gyrus above, the small crowded cells at the top of the pyramidal layer are less noticeable, the infra-granular pyramids become plumper, and more like those of the post-central area, and the stellate cells are less numerous. Posteriorly, the area becomes richer in cells in all layers, and the individual cells smaller—more nearly approaching the visual type; and here, again, the surface layer of crowded pyramidal cells becomes less obvious and finally disappears. Anteriorly the cortex, as it approaches the intermediate type "R," becomes less rich in cells, the infra-granular cells are smaller, and the lamination presents a less characteristic appearance.

Visual Area.

Extent and Boundaries: Visual A ▨.—This type follows the deeply infolded calcarine fissure, and is thus only partially visible on the surface. It is found on the posterior wall of the anterior limb of the calcarine fissure, on both walls of the posterior limb, and on the lower lip of the posterior half of the calcarine stem, ending within about 5 mm. of its anterior extremity. It sometimes only reaches to the base of the fissure, and sometimes extends on to the anterior wall, but it is not found on the surface on the anterior side of the fissure. Type "Visual B" bounds the area posteriorly.

Visual B.—Type "Visual B" covers the posterior pole of the hemisphere. On the mesial surface its anterior boundaries are type "Visual A," and imaginary lines drawn upwards in continuation of the superior extremity of the anterior limb of the calcarine fissure to the dorso-mesial border; and downwards from the inferior boundary of "Visual A," to the inferior occipital sulcus. It is bounded on the under surface by this sulcus, being found only on its superior wall; and it extends around the pole on to the dorsal surface for about 4 mm. Here it is bounded approximately by the rudimentary sulcus which is probably the homologue of the "Affenspalte" of the apes, but it frequently ends within about 1 mm. of the sulcus, merging into a type intermediate to the visual and temporal types; which is also its boundary between this sulcus and the inferior occipital.

Characteristics: Visual A (fig. 10).—The depth of the cortex is about 1 mm., the molecular layer measuring about 0·15 mm., the pyramidal layer about 0·4 mm., the granule layer 0·15 mm., the pallid zone about 0·15 mm., and the polymorph layer only about 0·1 mm. in depth. The pyramidal cells are small and closely packed together, giving a crowded appearance to this area. Stellate cells are present above the granules. The granule layer is a prominent feature. The cells are smaller than those forming the granule layer in the post-central and temporal types, and are closely packed together; thus presenting under a low power a very characteristic appearance. Above this layer a pallid zone may be observed, which is not, however, very pronounced, and is not so deep as the one described below. Large solitary cells are sometimes found in this position. Above this ill-defined pallid zone there is a suggestion of a duplicated line of granules in some scattered cells which lie at the bottom of the pyramidal layer. Below the granule layer there is a pallid zone about 0·15 mm. in depth, in which are scattered a few granules, pyramids, and polymorph cells. Both in this zone and above the granules are found the large solitary cells of Meynert—cells having a blunt triangular or roundish shape, and large nuclei, and measuring on an average 25 μ by 15 μ. The polymorph layer is shallow and crowded with cells.

*Visual B.** — This cortex is a little deeper in the pyramidal and polymorph layers than "Visual A," becoming gradually deeper as it approaches the intermediate areas between it and the temporal and parietal types. The cells of the pyramidal layer are still small, but are not so crowded as in "Visual A." There are large pyramids to be seen both above and below the granules, measuring about 20 to 30 μ by 5 μ. Scattered among the supragranular pyramids are a number of stellate cells, resembling those described in the temporal area. The granule layer is also less crowded, and the individual cells rather larger than in "Visual A." The pallid zone below the granules is not so conspicuous; and the zone above them, with the suggestion of duplication of the granule layer, has disappeared. The solitary cells of Meynert are not present, their place being taken by the infra-granular pyramids mentioned above.

Archipallium ▨. (*Olfactory Area.*)

Extent and Boundaries.—The olfactory area covers the under surface of the temporal lobe. It is bounded superiorly by the rhinal fissure, inferiorly and posteriorly by the hippocampal fissure, and anteriorly by that portion of the Sylvian fissure which lies below the rhinal fissure.

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* See footnote * on p. 495.

Characteristics (fig. 12).—The depth of the cortex is about 1·3 mm. It is thus considerably more shallow than the cortex of the neopallium, which measures, over most of its extent, about 2 mm. in depth. The molecular layer is comparatively deep, measuring about 0·3 mm. The pyramidal layer, which is about 0·6 mm. deep, is rather scantily filled with large angular cells, which stain well, and have long branching processes. The cells of the superficial layer are particularly noticeable. They are of characteristic irregular triangular or quadrate shapes, and often lie with the base towards the surface, with two, or even more, long branching processes passing upwards. Staining well, and lying closely crowded together, they form a conspicuous line at the top of the pyramidal layer. Below the pyramids a pallid zone can be seen measuring about 0·2 mm. There are a few granules scattered here and amongst the pyramids, but there is no definite granule layer. The polymorph layer is about 0·4 mm. deep.

The Hippocampal Fissure.—Several distinct types of cell arrangement can be observed within the hippocampal fissure. Their position will be best explained by referring to the diagrams. Fig. 4 is a section of the temporal lobe of a right hemisphere. On it will be seen the olfactory type of cortex between the hippocampal fissure and the rhinal fissure, and, externally, type "R" lying between the olfactory and the temporal types. Within the hippocampal fissure, and hidden from view on the surface by the basal ganglia, a narrow gyrus is shown, in section. The cortex on this gyrus was found to be of the same type as the limbic (*vide* fig. 13), though somewhat more shallow (perhaps owing to pressure within the fissure). The types *a*, *b*, *c*, *d*, figured in No. 14, are found in the positions marked *a* . . . *a*, *b* . . . *b*, etc., respectively on fig. 4, the last evidently corresponding to the stratum granulosum of the human brain.

*The Olfactory Tract.**—The molecular layer is deep and contains islets of small angular cells. The cells of the next layer are larger and are still very angular in shape; they have fine processes, stain well, and are rather closely crowded together. Under a low power it can be seen that this layer is arranged in a zigzag fashion. There are a number of irregularly scattered large angular cells below this layer. This structure seems to correspond to the tuberculum olfactorium described by Ramon y Cajal and Campbell.

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* See footnote * on p. 495.

Type "R" ▤.

If the lips of the Sylvian fissure are opened out, it will be seen that the anterior portion of both the superior and inferior walls form an operculum, and cover an area of cortex which is continuous with the cortex below the orbital sulcus anteriorly, and with that which lies directly above the rhinal fissure posteriorly. It is triangular in shape, the apex of the triangle being wedged between the walls of the Sylvian fissure about half way between its posterior extremity and the vallecula Sylvii. This corresponds to the floor of the Sylvian pit in the human fœtal brain before the temporal and fronto-parietal opercula have covered it up to form the Sylvian fissure. It is bounded within the fissure both anteriorly and posteriorly by small sulci, which lie at the base of the superior and inferior walls respectively. Histological examination shows that this cortex is similar to, and continuous with, the type which borders the olfactory tract anteriorly, and the olfactory area posteriorly. When, in the higher apes, the development of the frontal lobe pushes the fronto-orbital sulcus downwards and backwards until its posterior extremity joins the Sylvian fissure (thus forming, according to Elliot Smith, the anterior ramus of the complete Sylvian fissure), this cortex is pressed downwards and backwards within the Sylvian fissure. And, further, the fuller development of the temporal lobe may press the part bordering the rhinal fissure forwards—also within the increasingly operculated Sylvian fissure. It is thus probable that this type of cortex, which borders the olfactory area in the lemur, may be the homologue of the island of Reil in the higher anthropoids.

This area has been designated, for convenience, type "R." As it is impossible to show the whole extent of the area on the surface map (fig. 1), in fig. 3 a diagram of a transverse section of the Sylvian fissure has been given, to show the position of the submerged gyrus.

Extent and Boundaries.—This cortex covers (*a*) that part of the orbital surface which borders the olfactory bulb and tract; (*b*) the continuation of this area into the gyrus lying within the Sylvian fissure; (*c*) the portion of the temporal lobe which lies immediately above the rhinal fissure.

Characteristics.—The depth of the cortex is about 1·8 mm. The molecular layer is comparatively deep, measuring about 0·25 mm. (fig. 11). The cells of the superficial layer of the pyramids resemble those found in the same position in the olfactory area, but are smaller. That is to say, they are of irregular triangular or quadrilateral shapes, and frequently have more than one branching process passing upwards. In the remainder of the pyramidal layer the cells are less elongated in shape than the typical pyramidal cells of the neopallium. There is no definite granule layer, but a number of faintly staining granules are scattered below, and in the lower part of the pyramidal layer. Infra-granular pyramids, measuring about 30 μ by 15 μ, take the stain well, and form a fairly conspicuous line.

This area seems to be intermediate in type to the neopallium and the archipallium. On comparing fig. 11 (type "R") with fig. 12 (olfactory type) and fig. 9 (neopallium, temporal type), it will be seen that the cortex is deeper in type "R" than in the olfactory area, but more shallow than in the neopallium; and that in the deep molecular layer, and also in the form of the superficial layer of the pyramids, it resembles the olfactory type; but in other respects it follows the neopallial type.

Limbic Area ⊙ ⊙ ⊙.

Extent and Boundaries.—The limbic type forms a band around the whole extent of the corpus callosum, and extends along the narrow gyrus between the calcarine and hippocampal fissures, which forms a link between the upper and lower portions of the limbic lobe. (This posterior part of the area is only visible on the surface by the removal of the basal ganglia by which the upper portion of the hippocampal fissure is exposed.) The intercalary sulcus forms the superior boundary of this area, and an imaginary line continues it to the anterior limb of the calcarine fissures. Anteriorly it spreads into a band, about 5 mm. wide, around the anterior genu of the corpus callosum. The anterior limb of the calcarine fissure, and, as the area extends downwards in a narrow strip, the calcarine stem form its posterior boundaries, and inferiorly it is folded within the hippocampal fissure, as is shown on fig. 4.

Characteristics (fig. 13).—The cortex measures about 1·3 mm. in depth. It seems to be of low development, and cannot be readily divided into layers. The molecular layer is deep, measuring about 0·23 mm. The layer corresponding in position to the pyramidal layer of the neopallium is formed of blunt triangular or rounded cells, which take the stain poorly, and have no tendency to regular arrangement in columns. These cells form a layer about 0·8 mm. deep. Below it there is a layer of faintly-stained polymorph cells. Between the two layers is a scattered line of sharply-stained stellate cells measuring about 15 μ by 7 μ. A number of granules are scattered throughout the cortex, especially in the lower part of the pyramidal layer.

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4. Brodmann, "Beiträge zur histologischen Lokalisation der Grosshirnrinde," Mitteilung v, vi.
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Addendum.

All these drawings and sections were shown at the Physiological Society on January 25th, 1907 ; but we have delayed publication until the experiments performed by one of us, in conjunction with Professor Halliburton, had enabled us to map out the motor area. For we were uncertain of its extent in those regions where the giganto-pyramids are not found—namely, in the lower part of the motor area. Moreover, we found great difficulty in deciding upon the exact localisation of the visual area, for there is an undoubted difference in the structure of the cortex lying within the calcarine fissure and the circumjacent cortex on the mesial and external surface; which, although it shows by the fibre method a band which seems to be a definite line of Gennari, yet differs considerably in cell-structure from that within the calcarine fissure. The cortex within the fissure is less deep, and the pyramids and granules are more closely crowded together. This may quite probably be due to mechanical pressure within the fissure. But a more important difference is found in the fact that the solitary cells of Meynert lying within the fissure are more numerous, larger, and more typical in shape. In the surrounding region the cells, which are found in a corresponding position, form a more scattered line, are smaller, and are more pyramidal in shape. There is also a suggestion within the fissure of a pallid zone above the granules, and of a duplicated line of granules above this zone (*vide* fig. 10). This cannot be distinguished in the surrounding cortex. These observations find support in the fact that no movements were obtained by stimulation of the posterior pole. This experiment showed a marked contrast to the effects of stimulation of the pole in the ape. It may possibly be explained by the fact that the stimulus did not reach the solitary cells within the calcarine fissure. We have termed the cortex within the fissure "Visual A," and the surrounding area "Visual B." While on the one hand the line of Gennari seems to stretch over the whole of the area we have mapped out as "visual," on the other hand, for the reasons given above, we are unable to affirm whether the surrounding cortex corresponds to the "visuo-sensory" type of Bolton and Campbell or to their "visuo-psychic."

Since this paper was written we have received from Professor Brodmann, 'Beiträge zur histologischen Lokalisation der Grosshirnrinde, Fünfte Mitteilung': "Über den allgemeinen Bauplan des Cortex pallii bei den Mammaliern und zwei homologe Rindenfelder im besonderen.—Zugleich ein Beitrag zur Furchenlehre," and 'Beiträge zur histologischen Lokalisation der Grosshirnrinde,' VI. Mitteilung: "Die Cortexgliederung des Menschen," which contain an account of his researches on localisation in the lemur's brain. Essentially there is little difference between our results and those figured by Brodmann, except that he has defined more subdivisions of the cortical types; while we have found the change from one type to another in the neopallium to be so gradual that it is hardly possible in most cases to define an exact boundary line between them; and we have preferred, as stated in the paper, to leave blank spaces on the diagrams where the intermediate areas occur, as they seem too indefinite in this brain, both in characteristics and in extent, to allow an accurate representation of their areas to be given. Thus we have limited ourselves to mapping out the main types of the neopallium—namely, the motor, frontal, temporal, post-central, and visual types, to pointing out variations in these types, and to giving some description in the text of the intermediate areas. Apart from this, his diagram chiefly differs from ours in that we have given a broader band for the motor area, carrying it further back—especially in the lower part; and that we have not carried the visual area so far forward on the dorsal surface. Brodmann's researches were made with the brain of the *Lemur macaco,* while the species we have used were *Lemur brunneus, Lemur catta,* and *Lemur mongoz.*

FIG. 5.—MOTOR A.

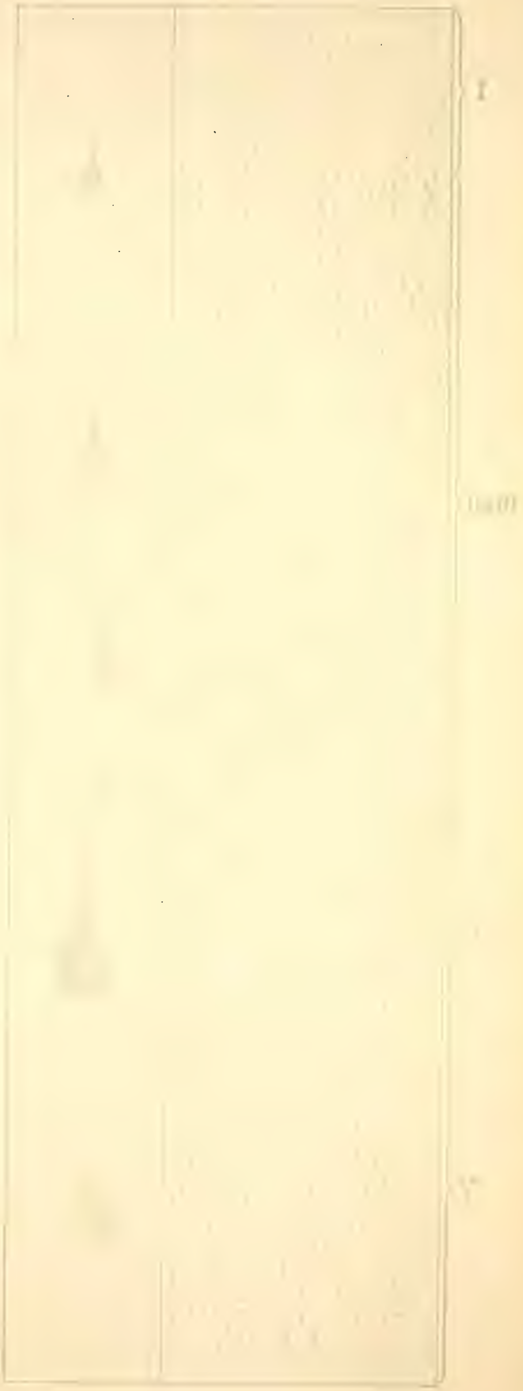

FIG. 6.—MOTOR B.

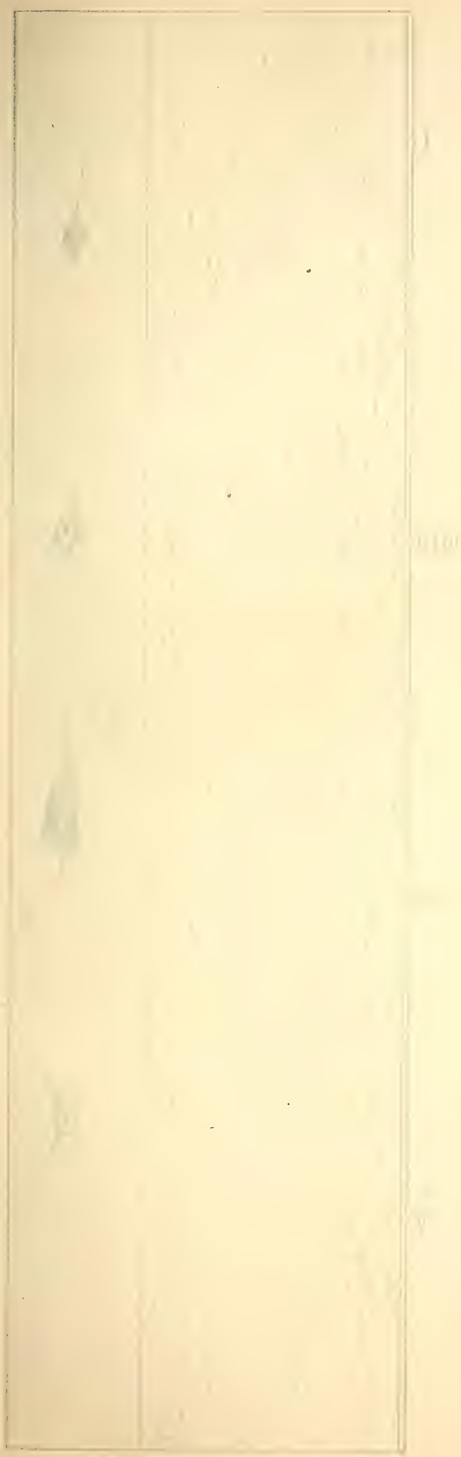

Fig. 7.—Frontal A.

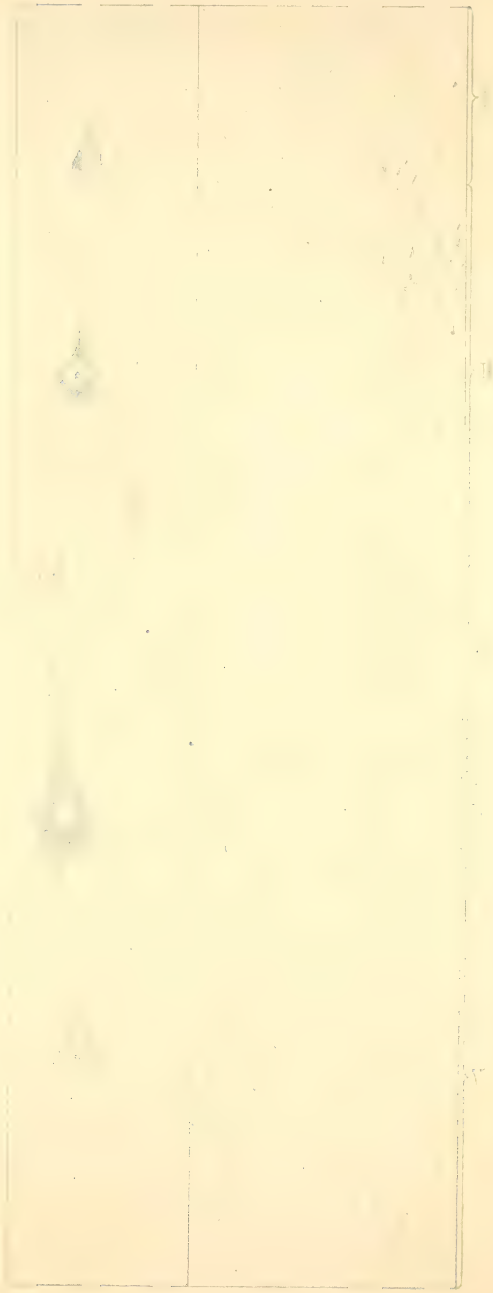

Fig. 8.—Post Central.

Fig. 9.—Temporal A.

Fig. 10.—Visual A.

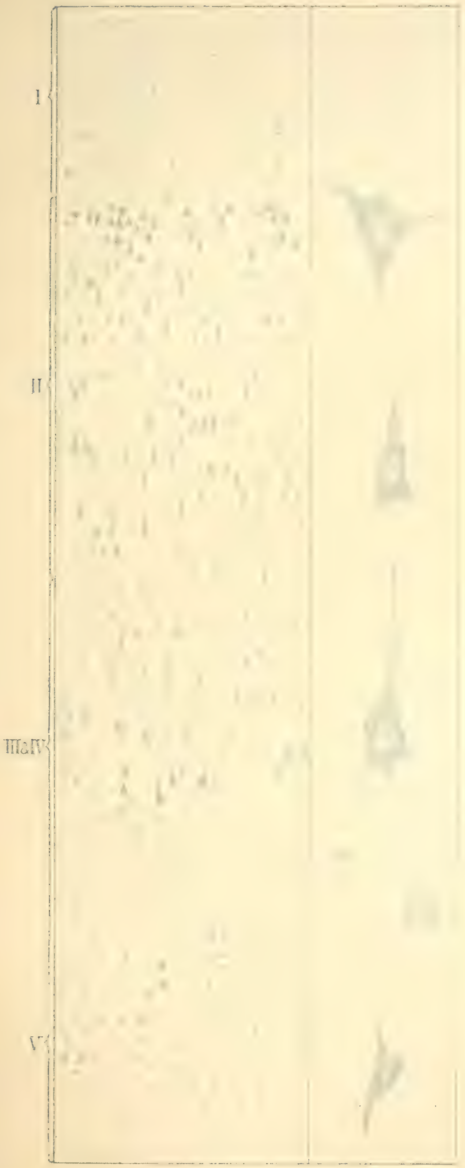

FIG. 11.—TYPE "R."

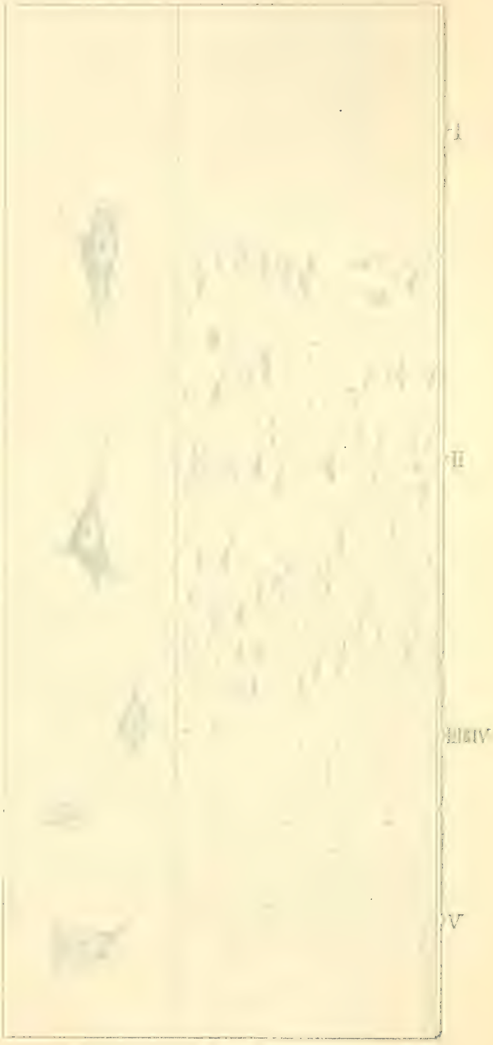

FIG. 12.—OLFACTORY.

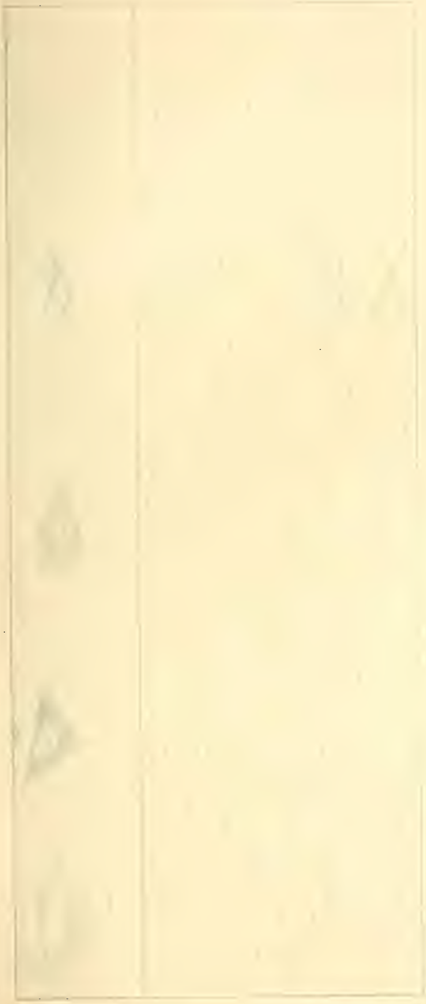

FIG. 13.—LIMBIC.

Hippocampal
a.
Hippocampal
b.
Hippocampal
c.

FIG. 14.—HIPPOCAMPAL AREA.

An Investigation on the Anatomical Structure and Relationships of the Labyrinth in the Reptile, the Bird, and the Mammal.

By Albert A. Gray, M.D., F.R.S.E.

(Communicated by John G. McKendrick, M.D., F.R.S. Received June 1, 1908.)

[Plates 19 and 20.]

The object of this paper is to give a description of the labyrinth of certain animals, illustrating the organ when it is viewed as a whole in examples chosen from the reptiles, birds and mammals.

The method of preparation employed was that devised by the writer and described in the "Labyrinth of Animals," vol. 1, p. 8. By the use of this method new facts have been brought to light, and these enable us to elucidate certain relationships in the anatomical structures which have hitherto been obscure and which anatomists are not agreed upon. The structures referred to are, the aqueduct of the perilymph, the perilymph recess and the round window. A special portion of the paper, therefore, has been directed to this aspect of the subject.

Before proceeding to describe the organs mentioned, the writer would like to thank very cordially those who have assisted him in obtaining material. Since some of the animals are rare and difficult to obtain, it will naturally be understood that the writer is very grateful to those who have given them to him for preparation, and he is glad to have this opportunity of thanking the Zoological Society of London, the Royal College of Surgeons of London, and the Hon. N. Charles Rothschild.

The Membranous Labyrinth of the Monitor (Varanus salvator).

(Plate 19, figs. 1, 2, and 3.)

In extracting the labyrinth of this reptile it was found possible to make a more complete preparation of the organ than was done in any of the reptiles examined hitherto. This has brought into light some interesting facts which help to elucidate considerably the relationship of the reptilian to the avian and mammalian labyrinths.

The organ measures 8 mm. in its greatest length from the junction of the superior and posterior canals to the tip of the cochlea. The *cochlea* measures 3·5 mm. in length from the oval window to the tip, and the tube of the cochlea measured at its base is 1·75 mm. in diameter. The reason why this measurement of the length of the cochlea appears to be so great relatively to the same measurement in the bird is owing to the fact that, in the reptile, the oval window is situated in the vestibule, while in the bird it is placed some little distance along the tube of the cochlea.

The *vestibule* measures 3·75 mm. in its greatest diameter, and the major axis of the oval window is nearly 1·5 mm. in length.

The *superior canal* measures 4·5 mm. in internal, and 6·5 mm. in external diameter. The greatest distance of the canal from the vestibule is 1·5 mm., and the diameter of the canal itself is 1 mm. The *posterior canal* has internal and external diameters of 4 mm. and 6 mm. respectively. The greatest distance of the canal from the vestibule is 0·5 mm., and the diameter of the canal itself is 0·75 mm.

The *horizontal canal* measures 3 mm. in internal, and 5 mm. in external diameter. The greatest distance of the canal from the vestibule is 1 mm., and the diameter of the tube of the canal itself is 0·75 mm.

In its general shape the labyrinth of the monitor resembles those of other reptiles; that is to say, it is roughly pyramidal. The canals do not present such a constantly curved outline as do those of the bird and the mammal, and in this respect also they are more like those of other reptiles. The perilymph space is well marked in the canals. The horizontal canal, *h.*, has no communication with the posterior canal, *p.*, at the point at which they cross, the monitor resembling the gecko in this respect and differing from all the other reptiles examined by the writer. There is no bridge connecting the upper surface of the saccule with the middle of the superior canal in the monitor, such as was found in the teguixin and the West African python.*

The vestibule in the monitor is very similar to that found in other reptiles, and needs but little remark. The saccule forms by far the larger portion of the vestibule. It is roughly cone-shaped, with the apex of the cone near the junction of the superior and posterior canals. It contains, and is almost entirely filled by, the large otolith mass, *o.s.*, which consists in this, as in other reptiles, of a collection of innumerable minute crystals held together by a semi-gelatinous envelope. The saccule is external to the utricle. The last-named cavity is very small, and is really only large enough to receive the openings of the three canals.

The oval window, *f.o.*, is situated at the lower and posterior portion of the saccule. It is oval in shape, resembling that of the mammals rather than that of other reptiles and birds. It is, indeed, a narrower ellipse than is found in the case of some mammals, such as the echidna and the

* Gray, *op. cit.*, vol. 2, pp. 214 and 222.

kangaroo. It is interesting to note that the oval window is situated further back relative to the saccule and the cochlea than in birds or mammals. Its posterior margin is almost in contact with the ampulla of the posterior canal.

The cochlea, *c.*, is slightly constricted after leaving the lower and inner portion of the saccule, and in the neck formed by this constriction lies the aqueduct of the perilymph, *d.p.* Passing downwards and inwards, the cochlea first bulges slightly and then tapers gradually to end as a cone. It is interesting to notice that, at the tip, the cochlea ends with a little nipple-shaped projection, exactly similar to that found in some of the ratite birds, such as the emu, ostrich and rhea.

The cartilaginous framework within the cochlea which supports the basilar membrane and the lagena, *l.*, is in the form of a long ellipse, the major axis of which is, of course, parallel with the long axis of the cochlea. It does not appear to be rotated upon itself to any very noticeable extent, the two limbs of the framework remaining anterior and posterior respectively throughout their whole course. In this respect the shape of the framework differs from that of birds; but it is similar in another respect to that of at least most birds in that, at its vestibular end, the cartilage bends sharply upon itself.

The ductus perilymphaticus, or aqueduct of the perilymph, *d.p.*, may be demonstrated very clearly in the labyrinth of the monitor, and shows very interesting relationships. It arises on the outer surface of the labyrinth at the junction of the cochlea with the saccule as a fairly wide tube. From this point it curves forward, then inwards, and finally backwards, being in contact with and encircling the neck of the cochlea, and reaches the inner posterior portion of that neck. The ductus perilymphaticus was traced by Retzius* to this point in an allied species (*Psammosaurus caspicus*), but he was unable to trace it further. Its further course is interesting, especially in view of the fact that it throws considerable light upon the relationship of the round window and perilymph recess to the cochlea as found in birds and mammals. After reaching the inner posterior surface of the cochlea, the aqueduct of the perilymph turns slightly downwards, and immediately widens out into a large cavity, roughly heart-shaped, and termed the perilymph recess, recessus perilymphaticus, or recessus scalæ tympani, *r.p.*

The perilymph recess has roughly five surfaces. The first of these surfaces is the uppermost, and is small, consisting merely of an opening *o.p.*, by means of which the perilymph recess communicates directly with the arachnoid space, and it therefore permits of free interchange between the cerebro-spinal fluid and the perilymph. The inner wall passes from above and within downwards and slightly outwards. It is roughly rectangular in shape, and its long anterior margin runs close to the tip of the cochlea. This surface is covered entirely by bone. The posterior wall runs from the posterior edge of the inner wall outwards and backwards. The anterior outer wall runs from the anterior edge of the inner surface backwards and outwards to meet the posterior wall below the ampulla of the posterior semicircular canal. Lastly the inferior anterior wall, which may be termed the floor of the cavity, looks downwards, forwards and outwards (in fig. 2 the organ is rotated a little clockwise, so that the floor looks downwards, whereas in reality it looks a little forwards and outwards as well). The floor is roughly oval or rectangular in shape, and is entirely uncovered by bone. It is, however, closed by a thin membrane, *f.r.*, and looks directly into the tympanum. This corresponds anatomically, and perhaps also functionally, to the round window of birds and mammals. But this portion of the subject will be referred to later in the paper.

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* Retzius, 'Das Gehörorgan d. Wirbelthiere,' vol. 2, p. 98.

Pigment is found in the labyrinth of the monitor, scattered in specks over the surface of the perilymph recess, including the membrane closing the round window. It is also found over the surface of the cochlea, and more particularly over the walls of the aqueduct of the perilymph. A few specks are found on the wall of the saccule which is adjacent to the cochlea. The distribution of pigment, therefore, is very similar in arrangement to that found in the lizard and teguixin. It is, perhaps, still more interesting to note that the distribution of pigment in the labyrinth of birds in those rare cases in which it is found, the ostrich, rhea and tinamou, is similar to that found in the teguixin, the lizard and the monitor. In the tortoise and the python it is either entirely absent or nearly so, whereas in the gecko it is found equally abundant over the whole surface of the labyrinth.

In addition to the large otolith mass already described as being found in the saccule, there is a string of small otoliths in the cochlea which runs along the basilar portion of the organ, and on reaching the lagenar portion the string of otoliths widens out into a spoon-shaped arrangement along the anterior wall.

Yet another otolith, *o.u.*, is found in the labyrinth of the monitor, and this one is of special interest since it forshadows in the reptile the otolith which is found in the bird. It is seen in that portion of the utricle which runs forwards to the ampullæ of the horizontal and superior canals, and lies internal to, and behind these ampullæ respectively. It is, therefore, in exactly the same position in which the otolith or otoliths (for there are sometimes two) are found in the utricle of the bird. But in yet another respect this otolith is similar to those found in the same position in birds. It consists of one comparatively large crystal and not of a collection of minute granules as does the large otolith in the saccule.

The Membranous Labyrinth of the Emu (Dromæus novæ-hollandiæ).

(Figs. 4 and 5.)

The labyrinth of the emu is, as might be expected, a large one, larger indeed, than that of any bird that has been examined, except the ostrich.

It measures 17·5 mm. in its greatest length from the uppermost point on the superior canal to the tip of the cochlea. The *cochlea* measures 4 mm. from the anterior margin of the oval window to the tip of the organ. The diameter of the tube of the cochlea immediately in front of the oval window is rather more than 2·5 mm.

The longest diameter of the *vestibule* is 4 mm. and the length of the major axis of the oval window is 2 mm.

The *superior canal* measures rather more than 8·5 mm. in internal, and 11·5 mm. in external diameter. The height of the vertex of the canal above the vestibule is 9·5 mm. and the diameter of the canal at the vertex is 1·25 mm. The *posterior canal* measures 5 mm. in internal, and 7·5 mm. in external diameter. The height of the vertex of the canal above the vestibule is 4·5 mm., and the diameter of the tube of the canal itself is 1·25 mm. The *horizontal canal* has internal and external diameters of 5 mm. and 7·5 mm. respectively. The height of the vertex of the canal above the vestibule is rather more than 3·5 mm. and the diameter of the tube of the canal itself at the vertex is 1·25 mm.

The labyrinth of the emu presents considerable similarity to those of the other ratite birds which have been examined by the writer, viz., the ostrich, the rhea and the apteryx. The similarity is much more pronounced between the emu, ostrich and rhea, than between any of these and the apteryx.

The semicircular canals in the emu are well curved, there being none of the angularity which is found to a certain extent in the apteryx alone among birds. The superior, *s.*, is the largest of the three canals and is of the "drooping" type. The perilymph space is well marked in all the canals and the cristæ acusticæ of the ampullæ are "simple" in character. There is no approximation to a channel of communication between the horizontal and superior canals such as is found in some of the carinate birds. In these respects the labyrinth of the emu agrees with those of the other ratite birds.

In the labyrinth of the emu, as in those of the ostrich and apteryx, there is no communication between the posterior, *p.*, and horizontal canals, *h.*, at the point at which they cross one another. This arrangement is different from that found in the rhea and in all the carinate birds hitherto examined, with the exception to be described in this paper, the penguin. The significance of this communication has already been discussed, from the evolutionary point of view,* but a few words may, with advantage, be added now. The channel is present in many reptiles, but not in all, *e.g.*, the gecko and monitor. In birds it was supposed to be invariably present until its absence was demonstrated by the writer in the ostrich and apteryx. In mammals, on the other hand, it has only been found to be present in a limited number of the polyprotodont marsupials and the insectivora. In such amphibia as have been examined, the frog and the toad, the channel was found to be present.

It is thus difficult to account for the facts as they are manifest in birds. In their origin from reptiles, birds may either have possessed this channel and it has been lost in the ratite branch, with the exception of the rhea; or they may not have derived it from the reptilian stock, it having been acquired by the carinate birds and by the rhea among the ratitæ. On these two views, suggested by himself, the writer formerly declined to express an opinion as to which was the more probably correct.† But, with the disposition of the canals in the emu, he is now rather inclined to the view that the channel of communication referred to has been acquired by the birds, and is not a remnant of reptilian ancestry. It is, of course, quite impossible to dogmatise, but in view of the fact that in the ostrich, emu, and apteryx, the channel is absent, and that that these are undoubtedly birds of an ancient type, the opinion above expressed seems the more probable, in spite of the fact that the rhea possesses the communication under discussion. Further, in support of this opinion, it should be pointed out that the communication is present in all carinate birds that have been examined by the writer, with the single exception of the penguin; and it is of considerable significance that the penguin is admittedly an archaic type of bird.‡ The writer has in course of preparation the labyrinth of the cassowary, and its examination will throw fresh light upon this point.

As regards the physiological significance of this channel of communication, if there is any such significance at all, it must be admitted that its function is obscure. The channel, when present, only allows of the passage of the perilymph from one canal to the other, the endolymph spaces of the two canals always remaining quite separate.

The cochlea, *c.*, of the emu is, in general, similar to those of the ratite birds.

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[*] Gray, *op. cit.*, vol. 2, p. 95.

[†] *Op. cit.*, vol. 2, p. 93.

[‡] 'Cambridge Nat. Hist.,' vol. 9, p. 94.

That is to say, it is short, and the tube is wide in proportion to its length. The lagena, *l.*, is large, and at the tip the cochlea ends in a little nipple-shaped projection which is peculiarly characteristic of the labyrinth of the ostrich and rhea. The tegmentum vasculosum, *t.v.*, that curious plexus of parallel venous spaces, is found in the emu, as it has been in all other birds hitherto examined, with the single exception of the ostrich. According to Retzius, it is found in at least some reptiles, and the present writer has found it in the apteryx. Its apparent absence in the ostrich is, therefore, most remarkable, and it may be that it is present in a modified form, though the writer was unable to find even a trace of it in three different specimens of the Masai ostrich. In the emu, as stated above, the tegmentum vasculosum is clearly marked, and has an appearance similar to that found in the rhea, but the parallel venous spaces are fewer in number, and rather wider apart than in the latter.

The recessus perilymphaticus, *r.p.*, that large cavity which opens out of the scala tympani of the cochlea, is well developed in the emu as in the other ratite birds. It is, however, more oval in shape than in the ostrich and rhea, in which it bears some resemblance to a sack with corners. On the lower and anterior surface of the perilymph recess is the round window, which is oval in shape, as in other birds and in reptiles. In the emu, the round window is of considerable size, larger, for example, than the oval window, *f.o.* At the opposite end, that is, at the upper posterior portion of the perilymph recess, is the aqueductus perilymphaticus, *d.p.* The latter leaves the perilymph recess directly and, after a short course of 1 or 2 mm., opens into the cranial cavity. Its disposition, therefore, is like that found in the ostrich, the rhea, the common fowl, the tinamou, and some other birds. In the crow, the night-heron, and many of the carinate birds, the aqueductus perilymphaticus opens out of the posterior wall of the perilymph recess, and instead of passing directly towards the cranial cavity, it curves upward in contact with the posterior wall of the perilymph recess for about 2 mm., and then opens into the cranial cavity.

Pigment is not found in the labyrinth of the emu, at least in sufficient amount to be seen by the eye, even when the organ is magnified to ten times its natural size. In this respect, therefore, it differs from those of the ostrich, the rhea and the tinamou.

Otoliths of considerable size are found in the emu; they occupy similar positions as do those in birds generally, and are similar to them in shape. In the vestibule there are two of these crystals, *o.*, and they lie in the utricle immediately behind, and internal to, the openings of the superior and horizontal ampullæ into that cavity. They are flattened from above downwards. The remaining otolith is found in the lagena, *h.*, and is, as is common in birds, saddle-shaped, with the concave surface facing downwards. Thus the otoliths of the emu are very similar in position and shape to those found in the rhea.

The Membranous Labyrinth of the Rhea (Rhea Americana).

(Plate 20, fig. 6.)

As the labyrinth of the rhea has already been described, there is no need to give a general description of it here. But for the sake of comparison a representation of a portion of the organ is shown.

It will be noticed that the otoliths, both in the vestibule and in the lagena, *o.* and *l.*, are very similar to those of the emu. The cochlea is more bulbous at the inner portion than is that of the emu, but the nipple-shaped termination of the organ is present, though it has been broken in the course of preparation; the place where it has been broken off is clearly seen.

The perilymph recess, *r.p.*, is large in the rhea, and is sack-shaped as in the ostrich, rather than oval as in the emu. The round window, *f.r.*, is shown at the lower, outer, and anterior portion of the perilymph recess; it is large and has the form of a long ellipse.

Pigment is present in the form of minute granules, and is fairly abundant in amount, thus giving a speckled appearance to the perilymph recess. It is almost entirely confined to the perilymph recess, the distribution being similar to that found in the tinamou, whereas in the ostrich pigment is found over the surface of the perilymph recess and also over the adjacent surface of the cochlea.

The Membranous Labyrinth of the Penguin (Spheniscus demersus).

(Figs. 7, 8, and 9.)

The labyrinth of the penguin is large, measuring 16·5 mm. from the vertex of the superior canal to the tip of the cochlea. The *cochlea* measures 4·5 mm. from the anterior margin of the oval window to the tip, and the tube of the cochlea is 2 mm. in diameter at the level of the oval window.

The *vestibule* measures 4 mm. in its greatest diameter, and the major axis of the oval window is 1·75 mm. in length.

The *superior canal* measures 7 mm. in internal, and 10·5 mm. in external diameter. The height of the vertex of the canal above the vestibule is 7·5 mm., and the diameter of the tube of the canal at the vertex is rather less than 1·5 mm. The *posterior canal* measures 4·5 mm. in internal, and 7 mm. in external diameter. The height of the vertex of the canal above the vestibule is rather less than 3·5 mm., and the diameter of the canal itself at its vertex is rather less than 1·5 mm. The *horizontal canal* has internal and external diameters of 5 and 7 mm. respectively. The height of the vertex of the canal above the vestibule is rather more than 3·5 mm., and the diameter of the canal itself at its vertex is 1·5 mm.

In the specimen of the labyrinth of the penguin which is represented in fig. 7, etc., air-bubbles unfortunately made their appearance in the celloidin in which the structures are embedded, and this interferes to a certain extent with the view of the parts. In addition to this the perilymph recess has been in part broken off.

In its general appearance the labyrinth of the penguin approaches most nearly to that of the red-throated diver among all the birds which have so far been examined. This is particularly noticeable in the cochlea, which terminates not as a rounded or bulbous extremity as in other birds, but comes rather to a tapering point. In both birds the superior canal, *s.*, is of the "drooping" type, and has the form of a long ellipse.

In the canals of the penguin, as in almost all birds, the perilymph space is well marked. The cristæ acusticæ in the ampullæ are "simple," there being only one crest in that portion of the neuro-epithelium which lies on the floor of the ampullæ. There is one peculiarity about the canals of the penguin which should be noted. There appears to be no channel of communication between the perilymph spaces of the horizontal and posterior canals at the point at which they cross, *x*. On close examination there does seem to be contact between the walls of the canals, but no actual channel from one to the other can be found in the specimen from which this description is taken. In this respect, therefore, the labyrinth of the penguin is somewhat similar to those of the ostrich, the emu, and the apteryx, and differs from all other birds which have been examined hitherto.

The cochlea, *c.*, of the penguin is of a rather primitive type in comparison with those of other carinate birds. The organ is proportionately somewhat short, the basilar portion being particularly small in extent.

The cartilaginous structures, *c.f.*, in the cochlea of the penguin deserve notice. It has been stated by some anatomists, that in the avian cochlea there are two cartilaginous plates which unite at their distal extremities in the region of the lagena, while each plate has its own termination at the vestibular end of the cochlea. This statement may be, and probably is, true in respect to some birds, but, as was pointed out by the writer, it is not true of all,* *e.g.*, the rhea. In this bird, the cartilaginous structure consists of one piece, which is bent sharply on itself at its vestibular end, while at the distal extremity, in the region of the lagena, the two approach one another and become fused into one. The ellipse formed by the two limbs of the cartilage is, of course, occupied by the basilar membrane. Furthermore, this elliptical cartilaginous framework is curved and also rotated upon itself to a certain extent, so that its concavity looks backward and upwards, the structure being therefore parallel with the curvature of the cochlea itself.

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* *Op. cit.*, vol. 2, p. 122.

In the penguin there is found almost exactly the same disposition of the cartilaginous framework as that just described in the rhea. That is to say, it does not consist of two plates of cartilage with different points of origin at the vestibular end of the cochlea, but of a single piece in the form of a long ellipse. At the vestibular and middle portions of the cochlea one of the limbs of the ellipse runs along the lower surface of the cochlea and separates the oval window from the large opening which gives access to the perilymph recess. The other limb runs along the upper surface of the cochlea, and through its substance pass those fibres of the cochlear nerve which are destined to supply the basilar membrane. Towards the lagena, however, the cartilaginous framework becomes rotated to a certain extent upon its long axis, so that the lower limb becomes posterior and the upper limb becomes anterior; the two limbs then become fused at the lagena.

This disposition of the cartilaginous framework within the cochlea of some birds is not difficult of explanation when it is remembered that in the reptiles the basilar macula is supported by a single circular or oval cartilaginous ring. The only change in the disposition of the cartilaginous framework which has taken place in the transition from the reptile to the bird is, that the circular ring has been drawn out into a rather long ellipse. Further, a certain amount of rotation round its own long axis appears to have occurred. Thus it comes about that in the proximal portion of the organ the limbs of the cartilage are superior and inferior, while at the lagenar end they are posterior and anterior.

The disposition of the cartilaginous framework in the cochlea of the bird is well seen in the photograph of the Cape gannet, and is undoubtedly the arrangement found in many other birds.*

With respect to otoliths in the labyrinth of the penguin, no conclusions can be drawn from the specimen obtained by the writer. It had lain for several years in spirit, and it is quite possible that the calcareous otoliths had been dissolved by some chemical agent produced during the long immersion in that fluid.

Pigment appears to be entirely absent from the labyrinth of the penguin;

* Gray, *op. cit.*, vol. 2, p. 134.

or, if present, the quantity must be so small in amount that it is invisible to the eye even when the organ is magnified to five or six times its natural size.

It is not possible to give here a satisfactory description of the perilymph recess and round window in the penguin, since in the specimen obtained by the writer the lower portion of the cavity was broken off, carrying with it the round window, if that opening exists in this bird. The destruction of the lower portion of the perilymph recess, however, has had one advantage: it shows clearly the large oval opening, *o.o.*, between that cavity and the scala tympani of the cochlea.

On the upper surface of the perilymph recess in the labyrinth of the penguin there is an opening, *d.p.*, in the wall of the cavity. This dehiscence is oval in shape and occupies the position of the aqueductus perilymphaticus (or aqueduct of the cochlea as it is sometimes termed) in other birds, such as the ostrich, emu, tinamou, etc. It corresponds exactly to the opening which is found in the same position in the monitor, and permits of intermingling of the perilymph with the cerebro-spinal fluid. In other words, the penguin does not possess an aqueductus perilymphaticus in the proper sense of the term, the channel being represented merely by an oval opening.

The Membranous Labyrinth of Echidna aculeata.

(Fig. 10.)

The labyrinth of the spiny ant-eater possesses the special interest which attaches to all the organs of the monotremes. Before going on to consider the relationships of the different parts, however, the measurements may be given.

The organ measures 11 mm. in its greatest length from the outermost point on the posterior canal to the innermost point on the cochlea. The whole diameter of the half whorl of which the *cochlea* consists is 6 mm. and the diameter of the tube of the cochlea itself just before its junction with the vestibule is 1·75 mm. The *vestibule* measures 3·5 mm. in its longest diameter and the major axis of the oval window 1 mm. in length.

The *superior canal* measures rather less than 4·5 mm. in internal, and 6 mm. in external diameter. The height of the vertex of the canal above the vestibule is 3·5 mm. and the diameter of the canal itself at the vertex is 0·75 mm. The *posterior canal* measures rather more than 2·5 mm. in internal, and 4 mm. in external diameter. The height of the vertex of the canal above the vestibule is 3 mm. and the diameter of the canal itself at the vertex is 0·75 mm. The *horizontal canal* measures 2·75 mm. in internal, and 4 mm. in external diameter. The height of the vertex of the canal above the vestibule is 2·5 mm. and the diameter of the canal itself at the vertex is 0·75 mm.

The cochlea is only possessed of half a turn and the straight distance from the anterior margin of the round window to the tip of the cochlea is 4·5 mm.

As stated above, there are numerous points of interest connected with the labyrinth of the echidna, some of which have been known previously, while others are now recorded. Further, certain errors, which inevitably arise from the attempt to reconstruct a whole larger structure from numerous fine microscopic sections, may be corrected when the organ is viewed as a whole.

Considered as a whole, the labyrinth of the echidna, while approaching rather more nearly the mammalian than the reptilian type, presents several remarkable similarities to the latter. Thus, as has been shown by previous anatomists, there is a lagena, *l.*, at the tip of the cochlea, and this structure is seen in fig. 10 as an oval cavity in that portion of the tip of the organ which lies nearest to the vestibule. It is supplied by a nerve, but it is not possible to say whether otoliths are present in the lagena or not. The specimen lay in rectified spirit for very many years, and it is quite possible that during that time they were dissolved if ever present. Since the processes for ordinary microscopic examination permit the contact of acids, otoliths, when present, are at once dissolved, so that the work of previous anatomists does not elucidate the matter. The question could be decided by preparing a comparatively fresh specimen by the writer's method, by which means otoliths are preserved, as is shown in the case of the reptile and the bird.

It has been shown by previous anatomists that the cochlea of the echidna only possesses a portion of a turn, but, judging from the figures drawn to represent the organ, a somewhat erroneous impression has been conveyed. As a matter of fact, the cochlea, *c.*, of the echidna presents the appearance of a larger portion of a circle than is usually depicted, most representations making it appear very similar to that of the platypus. In reality, the labyrinth of the echidna presents in this, as in other respects, a very considerable advance upon that of the platypus, in which there is only the slightest degree of curvature in the cochlea, with a bulbous knob on the tip of the organ.*

The aqueduct of the cochlea in echidna is a structure whose relationships are clearly reminiscent of its reptilian ancestry. Out of the base of the cochlea there opens, by a large oval aperture, an egg-shaped cavity, which corresponds to the perilymph recess of the reptile and the bird, and may be allowed to retain that name, *r.p.* On the outer surface of this perilymph recess is the round window, *f.r.*, and, as if further to express its reptilian character, the round window is markedly oval in shape. The long axis of the round window lies in the horizontal plane, as is the case in the reptile and the bird, and the membrane which closes the window looks directly into the tympanic cavity as in other animals.

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* Gray, *op. cit.*, vol. 2, p. 81.

On passing towards the cranial cavity the perilymph recess becomes narrowed to form the aqueduct of the cochlea, *p.a.*, which, in its further course towards the cranial cavity, lies above the jugular vein.

The vestibule in the echidna is also similar to that of the reptile except in the matter of size. The saccule forms the larger division of the vestibule, and in this animal, as in all mammals and reptiles that have been examined, the oval window abuts upon the saccule, the utricle being above and behind the latter. In the echidna the oval window, *f.o.*, is almost completely circular in shape, in this respect agreeing with that of the bird and many reptiles, and differing from that of the mammalia, though in certain of the marsupials there is a tendency towards the circular shape. In the echidna, therefore, as also in some reptiles and in birds, the oval window is round, and the round window is oval.

There is yet another primitive feature in the labyrinth of the echidna which has hitherto escaped recognition. That is the existence of a very definite recessus utriculi. In the echidna the recessus utriculi, *r.u.*, consists of a conical diverticulum from the utricle just where the ampulla of the horizontal canal opens into the utricle. The blunt and roughly cone-shaped recessus utriculi passes horizontally backwards and ends in a nipple-like projection just external to the middle of the outer wall of the utricle.

So far as the writer is aware, the presence of a recessus utriculi has not been suspected in any of the mammalia and certainly it has not been found in any of the mammals described by him. In the reptile and amphibian it does exist, but does not always form a prominence on the outer surface of the labyrinth, since the surrounding cavity of the perilymph rounds it off. In some birds, however, it forms a prominence in exactly the same position as in the echidna, and both in the latter and in the bird its floor is supplied by a nerve.*

The extent to which otoliths are present in the labyrinth of the echidna cannot be decided from the specimen in the writer's possession, for the reason above stated, viz., the very long period over which the preparation remained in spirit before the organ was extracted. In fig. 10 there certainly appear to be deposits like otoliths immediately internal to the oval window, but the fact that these are otoliths cannot be definitely stated. For reasons above given the ordinary examination of the organ by microscopic sections cannot throw any light upon the matter.

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* *Op. cit.*, vol. 2, p. 188.

In one respect, however, the vestibule of the echidna's labyrinth is distinctly more like that of mammals than that of at least the majority of reptiles. Its size, relative to the rest of the organ, is small, whereas in reptiles the vestibule is frequently the bulkiest portion of the whole labyrinth and occupies almost all the space contained by the planes of the three canals.

The semicircular canals of the echidna are very definitely mammalian in type (fig. 10, *s.h.p.*). They have entirely lost that angularity which is so characteristic of those of the reptile and of which vestiges are found in the platypus and the sloth.† Further, the junction of the superior and posterior canals is exactly similar to that found in mammals, with the exception of the platypus and sloth. In these two animals, as also in the reptiles, the two canals meet one another abruptly to form the common crus, whereas in the echidna and in mammals generally they approach one another with a gradual curve, so that when they unite a forked disposition of the union is produced.

The perilymph space in the canals is fairly well marked in the echidna, a feature which is invariable in the reptiles hitherto examined, but rather the exception in the mammals. The evolutionary significance of the perilymph space in the canals has already been discussed.‡ It was suggested that the original condition in mammalia presented the appearance of a perilymph space of considerable dimensions, as large, for example, as the endolymph space. In the very great majority of mammals, however, this space in the canals has been very nearly obliterated, but is still present in the primates, including man, in some of the marine carnivora, and in the edentates. Its existence in the echidna lends support to the view that originally the mammalian stock retained this space, inherited from their reptilian ancestor. The space is always present in birds, so far as present investigations have shown. In all animals which possess the space, it is almost entirely confined to the concave portion of the canal.

The white deposits seen in the canals in fig. 10 may be calcareous in character, but they can hardly be considered true otoliths, since, placed as they are at the vertices of the canals, they could have no physiological function similar to true otoliths; for there is no nerve supply to any portion of the canals except the ampullæ. The deposits seen in fig. 10 may be the result of the long sojourn of the head in alcohol before it was given to the writer for preparation of the labyrinths; and the deposits seen in the cochlea and vestibule may possibly be explained in the same way.

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* Gray, *op. cit.*, vol. 2, p. 81, and vol. 1, p. 159.

† Gray, *op. cit.*, vol. 1, p. 24.

In comparing the labyrinth of the echidna with that of the platypus, it must first be noticed that there are greater differences than has hitherto been supposed. The considerable increase in the curvature of the cochlea of the echidna, as compared with that of the platypus, has already been mentioned, and need not be referred to further. But in the canals also the disposition of the structures is very different in the two animals. As was shown above, the canals of the echidna are curved in outline and completely mammalian in type. In the platypus, on the other hand, the superior canal is noticeably angular and it meets the posterior canal abruptly, both of which features are characteristic of the reptilian labyrinth. The specimen of the labyrinth of the platypus obtained by the writer was a poor one, owing to the presence of hæmorrhagic opacities, and it cannot be determined whether that labyrinth possesses a recessus utriculi or not. The aqueduct of the cochlea also was removed in the labyrinth of the platypus, so in this feature also, comparison cannot be made between the labyrinths of the two animals. But in every other feature, at any rate so far as macroscopic evidence goes, the labyrinth of the echidna, though possessing many distinctive reptilian features, is clearly a more definite advance towards the mammalian type than is that of the platypus.

The Relationships of the Aqueduct of the Perilymph, the Perilymph Recess, and the Round Window to one another, and to the Cochlea.

In the labyrinths of the animals just described, certain facts are elicited which throw considerable light upon the difficult question of the relationship of the structures mentioned above. It is still a matter of doubt, in spite of the careful investigations of Hasse, Retzius, Harrison, Gaupp, Versluys and others, as to what particular structure, for example, in birds and reptiles corresponds to the round window of the mammals. The difficulty has, of course, arisen from the fact that investigations have hitherto been made by means of the examination of numerous serial microscopic sections or by casts of the organ, and it is not surprising, therefore, that differences of opinion exist in regard to the relationships of the different structures. By the method employed in making the preparations from which the descriptions in these pages are taken, some at least of the causes of error are eliminated.

In general it may be said, in respect to the relationships of the aqueduct of the perilymph, the perilymph recess, the round window and the cochlea, that in the reptile the condition is the most complicated, in the bird rather less so, and in the mammal it is comparatively simple. Further, the writer ventures to think that if the structures are viewed as a whole, the process by which this simplification has taken place may be traced in its various stages.

In the monitor the aqueduct of the perilymph opens out of the outer surface labyrinth at the point where the cochlea leaves the saccule. It curves first forwards then inwards and then backwards, thus encircling the neck of the cochlea. It then passes a little backwards and downwards and dilates into the large perilymph recess as described above. The anterior wall of the perilymph recess at its upper and outer part lies in contact with the posterior wall of the cochlea. In the bird there is no sign of the aqueduct of the perilymph surrounding the neck of the cochlea; all the portion of the aqueduct from its commencement to its dilatation into the perilymph recess appears to have become obliterated. But, as though to compensate for this, the adjacent walls of the cochlea and perilymph recess have become fused and an opening appears in this wall by which the perilymph fluid passes direct from the scala tympani into the perilymph recess. This is the condition found in the bird and it is also found in some reptiles such as the alligator.* The perilymph recess remains in other respects similar to that of the reptile. That is to say, it has the large round window (which is really oval both in the bird and reptile) at its lower, outer and anterior portion, and on its upper and inner extremity there opens a very short channel which corresponds to the dehiscence which occupies the same place in the monitor and through which the perilymph in the recess mingles with the cerebro-spinal fluid. Indeed, among the birds examined by the writer this structure still retained its reptilian form in the case of the penguin.

The confusion which has arisen as to what structure in the reptile and bird really represents the round window of mammals has undoubtedly arisen on account of the presence of the opening between the wall of the cochlea and the perilymph recess. Since it has hitherto been supposed that mammals do not possess a perilymph recess, it was naturally assumed that the opening from the cochlea into that cavity corresponded with the round window of the mammals. Now this opening occupies pretty accurately the position of the round window in mammals, but in reality it cannot be said to represent the round window of the bird and reptile at all. As stated above, the round window of these latter lies at the lower and anterior portion of the perilymph recess and looks directly into the tympanum. The round window of the reptile ultimately comes in mammals to occupy the position of the opening from the cochlea into the perilymph recess, and the process by which it does so is illustrated by a transition stage which may be seen in the case of the echidna, and which has hitherto escaped recognition. Though it has not hitherto been suspected, the echidna possesses a definite perilymph recess which communicates with the scala tympani of the cochlea by an oval opening corresponding exactly to that which exists in the same position in birds and in some reptiles, *e.g.*, the alligator. The perilymph recess of the echidna is not so large as in the bird or reptile, and the neck which unites it to the cochlea is not relatively quite so constricted as in these divisions of the vertebrata. Its reduction in size is owing to the fact that it has become a shallower pouch, the sides of the sack having become gathered up as it were. Thus the sack assumes the shape, roughly speaking, of an egg with its long axis horizontal, and that portion of the aqueduct of the perilymph which unites the perilymph recess with the cranial cavity in birds and reptiles tapers off from the posterior end of the perilymph recess in the echidna. Further, this portion of the aqueduct becomes a much longer tube owing to the deposition of bone round about it.

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* Retzius, *op. cit.*, vol. 2, p. 121.

By the same process the round window, which in reptiles and birds occupies only the lower portion of the outer wall of the perilymph recess, becomes raised up in the echidna and occupies the whole breadth of the outer wall of that cavity. It is interesting to note, moreover, that the round window in the echidna still retains the oval shape as seen in the reptile and the bird. Further, by the same process of raising of the round window, the anterior margin of the latter comes almost into contact with the wall of the cochlea at the outer margin of the oval opening between the cochlea and the perilymph recess.

The next step is seen in the kangaroo and wallaby. The oval opening between the scala tympani of the cochlea and the perilymph recess has become much wider, so much so indeed, that the floor of the cochlea sweeps gradually round to join the walls of the perilymph recess. Thus the latter cavity now forms a cone-shaped bulging on the floor of the scala tympani, there being little or no distinction as to the line at which the cochlea ends and the perilymph recess begins. The cone-shaped bulging, which is all that is now left of the latter cavity, has, of course, its base in the cochlea wall, while its apex tapers off into the aqueduct of the perilymph, passing inwards and backwards to open into the cranial cavity.*

By this process of the merging of the perilymph recess into the floor of the scala tympani, it is clear that the round window may be said now to open into the scala tympani, for the bulging on the floor of the latter is now all that is left of the perilymph recess.

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* Gray, *op. cit.*, vol. 2, p. 55.

The next stage need only be mentioned, for it consists merely in the reduction of the sharp cone-shaped bulging to a slight round bulging, and is found in many mammals, such as the carnivora and ungulates.*

The last stage is that found in the primates, including man. In it the bulging on the floor of the scala tympani has quite disappeared, its walls having been gathered in, as it were, until they are flush with the rest of the floor of the scala tympani. With this gathering-in process the round window must of necessity come to occupy a position in the outer wall of the scala tympani, and similarly the aqueduct of the perilymph comes to open out quite abruptly from the floor of the scala tympani. In the primates, the aqueduct of the cochlea is almost capillary in calibre throughout the earlier part of its course.†

When the labyrinths of reptiles, birds, and mammals are investigated in this way, it is not difficult to see why misconceptions should have arisen as to which structures in one division correspond with the structures in the other divisions. Thus, what is known as the ductus perilymphaticus in the reptile does not exist in the bird and mammal so far as present investigations show, and, conversely, the ductus perilymphaticus of mammals and birds is represented merely by an oval dehiscence in the roof of the perilymph recess of reptiles.

Again, the round window of man and the primates, with most other mammals, corresponds really to two structures in echidna, birds and reptiles, these two structures having come to coincide in the primates, etc. From the point of view of evolution the round window of man and most mammals corresponds with the opening low down in the perilymph recess of birds and reptiles, which is closed by a membrane. But as regards its position relative to the cochlea, the round window of man and mammals corresponds with the oval opening which exists between the cochlea and the perilymph recess of the echidna, the birds, and some reptiles.

Further, by tracing the labyrinth through its changes in reptiles, birds, and mammals by the means of investigation employed, the process by which the ductus perilymphaticus comes to open out of the cochlea is made clear. For it is to be noted that this aqueduct is found in the amphibians before the cochlea has made any appearance as a separate cavity, it being represented only by the macula basilaris. In this division, of course, the ductus perilymphaticus opens out of the vestibule.

It is somewhat difficult to describe these changes in words, and a series of diagrams has therefore been drawn up to show the matter more clearly.

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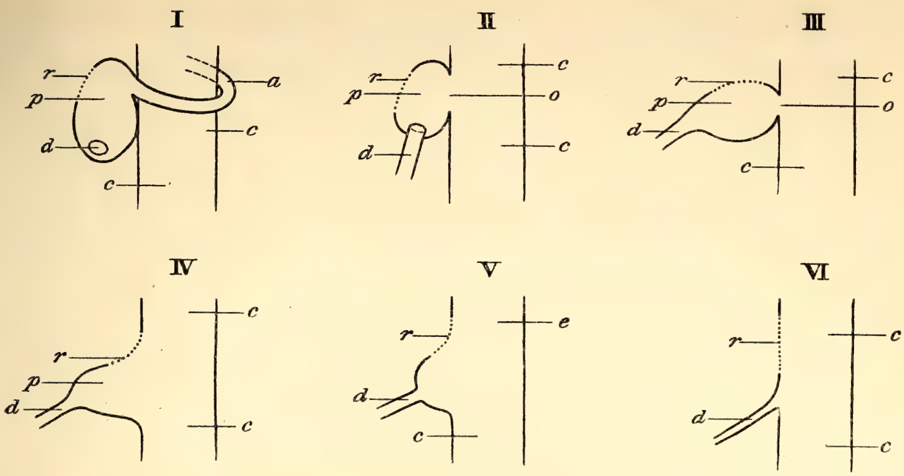
* Gray, *op. cit.*, vol. 1, pp. 91, 137, etc.

† Gray, *op. cit.*, vol. 1, p. 33.



Scheme of the Arrangement of the Ductus Perilymphaticus, etc., in Reptiles, Birds, and Mammals.

Fig. I.—Reptile, Monitor.—*a.*, aqueduct of perilymph. *c.c.*, cochlea. *p.*, perilymph recess. *d.*, dehiscence on roof of perilymph recess through which the perilymph is in direct communication with the cerebro-spinal fluid. This dehiscence becomes drawn out into the form of a short tube in the bird and a long tube in the mammal, and in these two divisions is termed the aqueduct of the perilymph. *r.*, round window, closed by a membrane, represented by a dotted line.

Fig. II.—Bird, Rhea, etc.—*o.*, oval opening between scala tympani of cochlea and perilymph recess ; it is not closed by a membrane. The other letters correspond with those in fig. 1, but it is to be noted that the oval opening, *d.*, in the reptile becomes the tube, *d.*, in the bird. The oval opening, *o.*, is present in all birds hitherto examined and in at least some reptiles, *e.g.*, alligator.

Fig. III.—Echidna.—The letters correspond with those of figs. I, II, etc.

Fig. IV.—Marsupial, Kangaroo.—The letters correspond with those of the other figures.

Fig. V.—Ungulates, Carnivora, etc.—The letters correspond with those of the other figures.

Fig. VI.—Primates, including Man.—The letters correspond with those of the other figures.

DESCRIPTION OF PLATES.

Plate 19, Fig. 1.

The Left Membranous Labyrinth of the Monitor, *Varanus salvator*, viewed from the outer aspect. × 5. The organ is rotated slightly counter-clockwise. The superior canal, *s.*, is to the left ; the posterior, *p.*, to the right ; and the horizontal, *h.*, is seen between them. The horizontal canal has no communication with the posterior canal at the point at which they cross. The large oval saccule is seen filling to a great extent the space between the canals, and the otolith apparatus, *o.s.*, occupies a large portion of the saccule. The single crystalline otolith of the utricle, *o.u.*, is seen internal to and behind the ampullæ of the horizontal and superior canals respectively. The oval window, *f.o.*, is seen opening into the lower posterior portion of the saccule below the arch of the horizontal canal. Immediately to the left of the oval window the aqueduct of the perilymph, *d.p.*, is seen opening out of the labyrinth at the junction of the saccule and cochlea, and passing round in front of the cochlea, then disappearing behind the inner wall of the latter, and finally reappearing at its posterior border to dilate into the large perilymph recess, *r.p.*, which lies to the right of the cochlea in the plate. The oval surface at the lower and outer portion of the perilymph recess is the round window, *f.r.*, and it will be noticed that it, in common with the other walls of the perilymph recess, is speckled with pigment. Pigment is also seen over the aqueduct of the perilymph and, more scantily, over the lower portion of the saccule and over the anterior wall of the cochlea, *c.*

PLATE 19, FIG. 2.

The Left Membranous Labyrinth of the Monitor, *Varanus salvator*, viewed from the inner aspect and a little below. × 8. The ampulla of the posterior canal, *a.p.*, is to the left, and it will be noticed that the crista acustica is "complex," that is, it has three crests, as is also the case in many birds. The aqueduct of the perilymph, *d.p.*, is seen running backwards over the inner surface of the neck of the cochlea, and then dilating into the large perilymph recess, *r.p.*, the anterior wall of which is in contact with the posterior wall of the cochlea, *c.* The cochlea is seen containing the oval cartilaginous framework and the otoliths, as described in the text. The little nipple-shaped termination to the cochlea is shown, and is similar to that found in most of the ratite birds.

l., lagena.
f.r., fenestra rotunda.
g., inferior angle of posterior canal.
a.p., ampulla of posterior canal.
p., superior angle of posterior canal.
o.s., otolith of saccule.
s., superior canal.
o.u., otolith of utricle.

PLATE 19, FIG. 3.

The Left Membranous Labyrinth of the Monitor, *Varanus salvator*, viewed from the inner aspect and slightly in front. × 8. Taken by reflected light. The oval opening, *o.p.*, on the upper surface of the perilymph recess, *r.p.*, is shown. It is by means of this opening that the perilymph communicates with the cerebro-spinal fluid. The opening corresponds to the aqueduct of the perilymph of birds and mammals. The other parts have already been described in figs. 1 and 2.

p., posterior canal.
s., superior canal.
o.s., otolith of saccule.
a.p., ampulla of posterior canal.
d.p., ductus perilymphaticus.
c., cochlea.
r.p., recessus perilymphaticus.
o.p., opening on upper surface of perilymph recess.

PLATE 19, FIG. 4.

The Right Membranous Labyrinth of the Emu, *Dromæus novæ-hollandiæ*. × 5 *ca.* The organ is viewed from the outer aspect and in front, and is rotated clockwise to the extent of about 20°. The superior canal, *s.*, is of the "drooping" type. There is no communication between the arches of the horizontal, *h.*, and posterior canals, *p.* The cristæ acusticæ in the ampullæ are "simple." The footplate of the columella has been left in position in the oval window, *f.o.* The large egg-shaped perilymph recess, *r.p.*, is seen behind the cochlea, *c.* The round window is barely visible. On looking through the perilymph recess to its upper and inner wall the narrow opening of the aqueduct of the perilymph, *d.p.*, is seen. The black patches on the walls of the perilymph recess are due to hæmorrhages. No pigment is visible. The nipple-shaped termination to the cochlea is seen below the lagena, *l.*

a.p., ampulla of posterior canal.

Plate 19, Fig. 5.

Portion of the Right Membranous Labyrinth of the Emu, *Dromæus novæ-hollandiæ.* × 10½ *ca.* Taken by transmitted light and viewed from the outer aspect. One of the otoliths of the utricle is seen as a sharply defined black structure, *o.*, at the top right-hand corner. The parallel venous spaces of the tegmentum vasculosum, *t.v.*, are well shown. The helicotrema, *h.c.*, is seen as an oval opening about three-quarters of the way along the cochlea, *c.*, to the right, with a large vein passing through it. A saddle-shaped otolith is present in the lagena, *l.* The disc-shaped footplate of the columella is seen in its natural position in the oval window, *f.o.*, and it is to be noted that the latter is situated in the cochlea some little distance from its beginning, and is in this respect different from those of the reptiles and mammals. The perilymph recess, the round window, and aqueduct of the perilymph are seen as described in Plate 4, but are more highly magnified.

Plate 20, Fig. 6.

Portion of the Right Membranous Labyrinth of the Rhea, *Rhea americana.* × 12. Taken by transmitted light and viewed from in front and slightly below. The cristæ acusticæ in the ampullæ are "simple." The otolith of the utricle, *o.*, is seen as a sharply defined black mass internal to and behind the ampullæ of the superior and horizontal canals, and is very similar to the same structure as found in the emu. In the cochlea the tegmentum vasculosum, *t.v.*, is shown and it will be noticed that the parallel venous channels are closer together and more numerous than in the emu. The columella has been removed from the oval window and the latter is more elliptical in shape than is the case in the emu. The large perilymph recess, *r.p.*, is not so definitely oval as in the emu, but is very similar to the perilymph recess in the monitor. The round window, *f.r.*, is large, occupying the whole of the floor of the perilymph recess : it is elliptical in outline and the membrane which closes it is richly pigmented. The walls of the perilymph recess are also pigmented. A saddle-shaped otolith is seen in the lagena, *l.* The little nipple-shaped termination to the cochlea has been broken off.

Plate 20, Fig. 7.

The Right Membranous Labyrinth of the Penguin, *Spheniscus demersus.* × 5. The organ is viewed from the inner aspect and is rotated a little counter-clockwise. Numerous small air-bubbles are present in the vestibule and a portion of the canals. The cristæ acusticæ in the ampullæ are "simple." It will be noticed that the lagenar portion, *l.*, is relatively large. The perilymph recess is seen to the right of the cochlea, *c.*, and there is an oval opening, *d.p.*, on the upper posterior surface of the cavity. This opening corresponds with that found in the same position in the monitor and has not been found in any other bird hitherto examined. It is, however, represented in other birds by a short tube, the aqueduct of the perilymph, as described in the text.

s., superior canal.
h., horizontal canal.
p., posterior canal
c.f., cartilaginous framework of cochlea.

PLATE 20, FIG. 8.

The Right Membranous Labyrinth of the Penguin, *Spheniscus demersus.* × 5. The organ is viewed from the outer aspect, above and in front. As the arch of the horizontal canal passes under that of the posterior canal at *x* it comes into contact with the latter, but there is no actual channel of communication between the two canals.

c., cochlea. *p.* posterior canal.

PLATE 20, FIG. 9.

Portion of the Right Membranous Labyrinth of the Penguin, *Spheniscus demersus.* × 10 *ca.* Viewed from behind and below. The lower part of the perilymph recess has been removed along with the round window (if present in this bird). The oval opening, *o.o.*, from the cochlea, *c.*, into the perilymph recess is clearly seen, and is bounded along one border by the lower limb of the cartilaginous framework, *c.f.*, in the cochlea. The bending of this cartilaginous framework is seen at the vestibular end of the cochlea. The tegmentum vasculosum, *t.v.*, has been to a considerable extent destroyed, but a fragment may be seen at the vestibular end of the cochlea. *l.*, lagena.

PLATE 20, FIG. 10.

The Right Membranous Labyrinth of the Spiny Ant-eater, *Echidna aculeata.* × 6. Viewed from the outer aspect and below. The semicircular canals are well curved and similar to those of other mammals, while differing from those of the platypus (see text). The perilymph space is fairly well marked in the canals and can be traced easily round the whole circumference. The cone-shaped recessus utriculi, *r.u.*, is seen lying between the ampulla of the horizontal canal, *h.*, and the outer wall of the utricle ; and the nerves supplying the ampullæ and recessus utriculi are also seen. The oval window, *f.o.*, is almost circular in shape and looks directly into the saccule. Immediately to the left of the oval window the perilymph recess, *r.p.*, may be seen opening out of the cochlea as an egg-shaped cavity, and the round window, *f.r.*, is seen on its outer surface as an oval opening. At its posterior extremity the perilymph recess again narrows down and tapers off into the aqueduct of the perilymph. The latter channel only occupies a portion of the irregular mass at the lower left-hand corner of the plate, a portion of the jugular vein, *j.*, being also present in the mass. In this mass, however, the aqueduct of the perilymph, *p.a.*, is seen on the right side as a somewhat lighter band. The cochlea, *c.*, is well curved, and the lagena, *l.*, may be seen as a kidney-shaped structure at the tip of the cochlea on the side nearest to the vestibule. The irregular slit at the tip of the cochlea is an artefact produced by the long immersion of the head in alcohol. The white deposits in the different parts of the organ are not to be looked upon as otoliths unless their presence in these situations is confirmed by further examinations (see text).

s., superior canal.	*h.*, horizontal canal.
p., posterior canal.	*n.s.*, nerve to ampulla of superior canal.

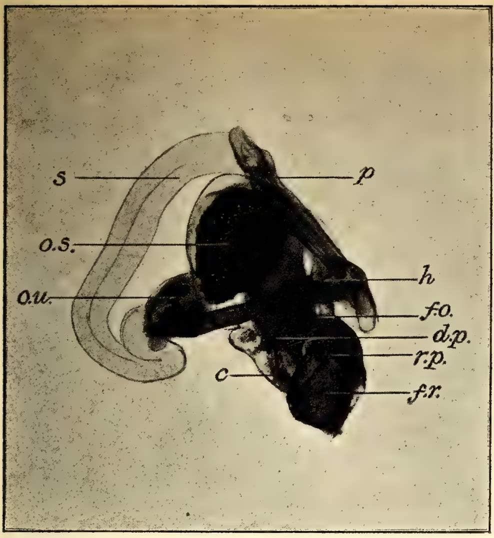

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FIG. 1.

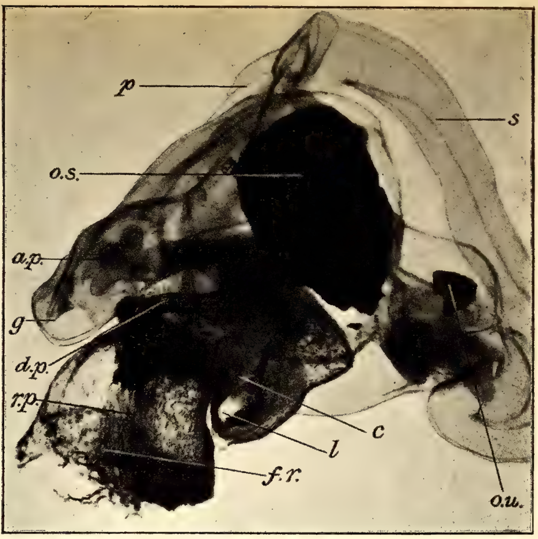
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FIG. 2.

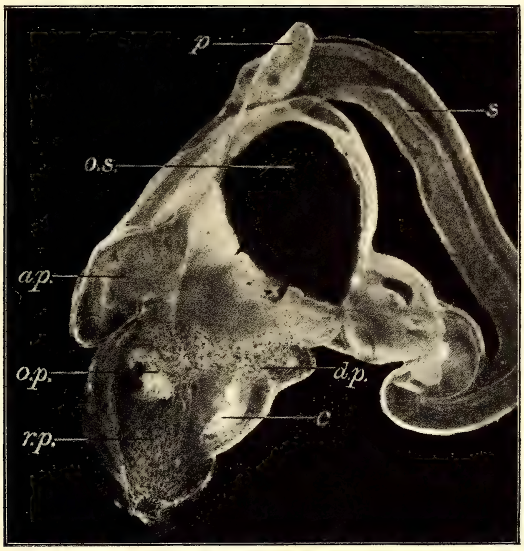
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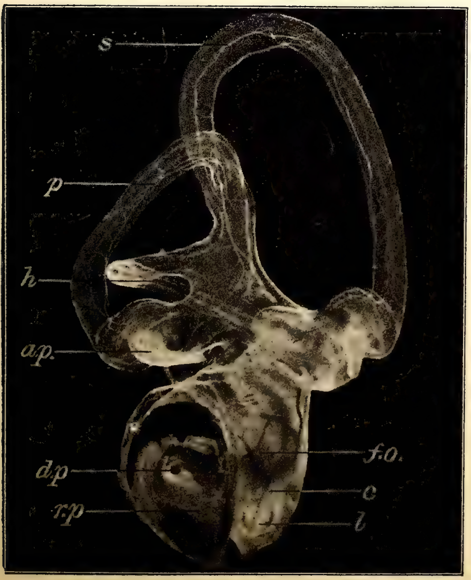
FIG. 3.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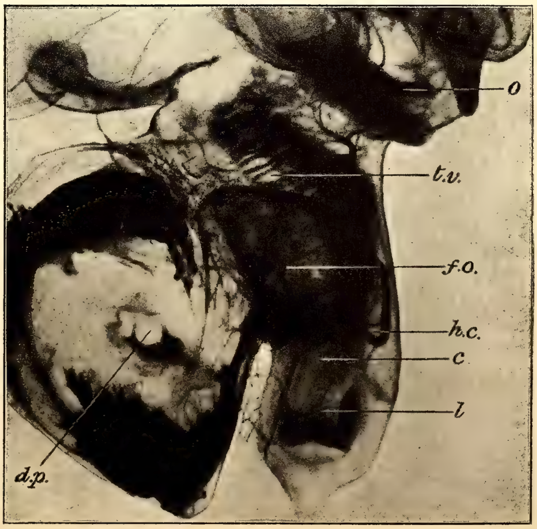
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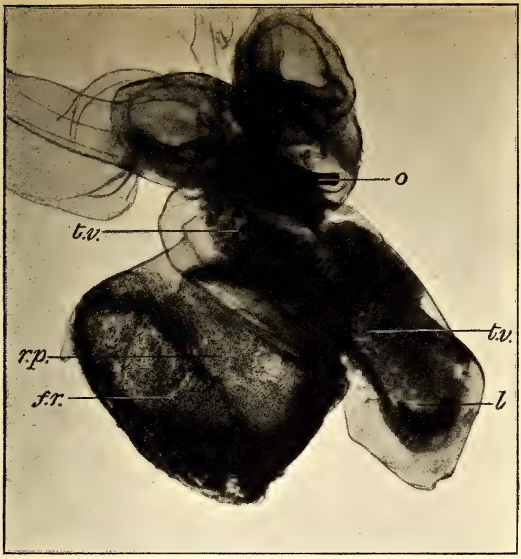
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r.p.
f.r.
l

FIG. 6.

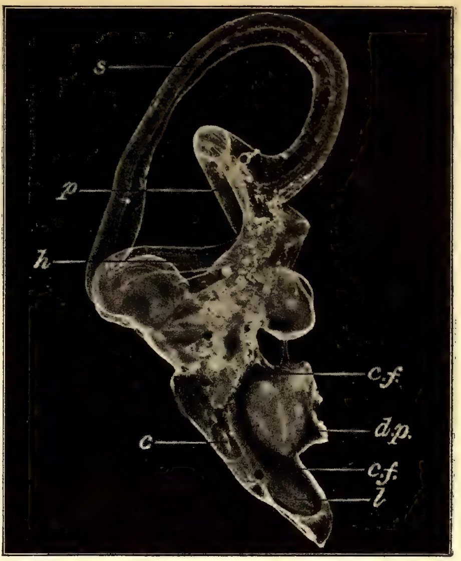
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FIG. 7.

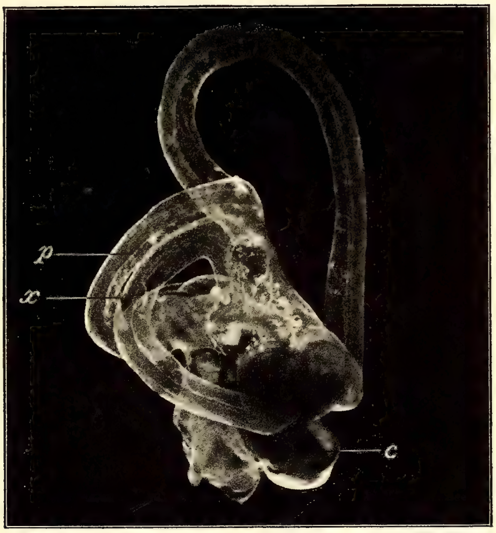


FIG. 8.

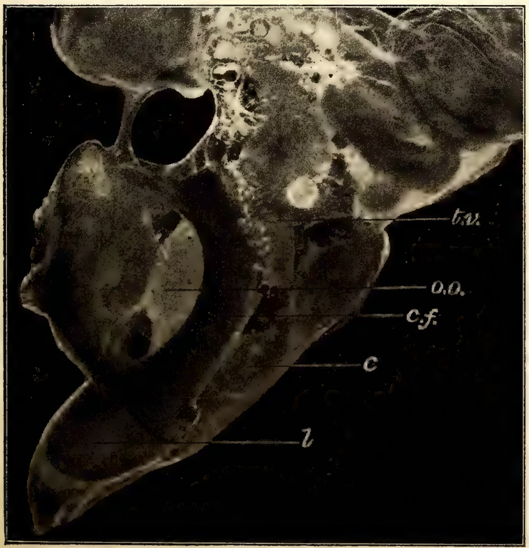
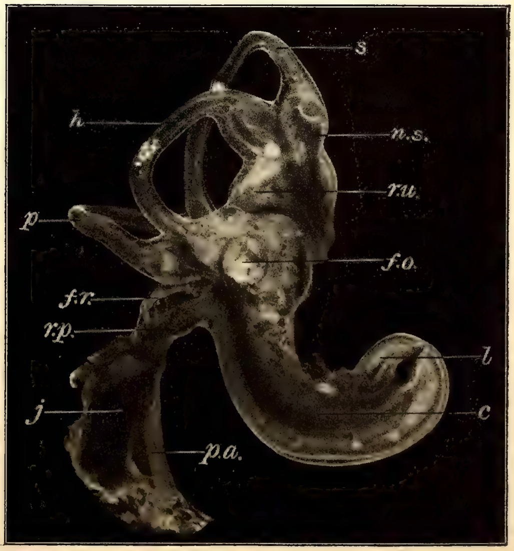


FIG. 9.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f.r.
r.p.
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p.a.

FIG. 10.

OBITUARY NOTICES

OF

FELLOWS DECEASED.

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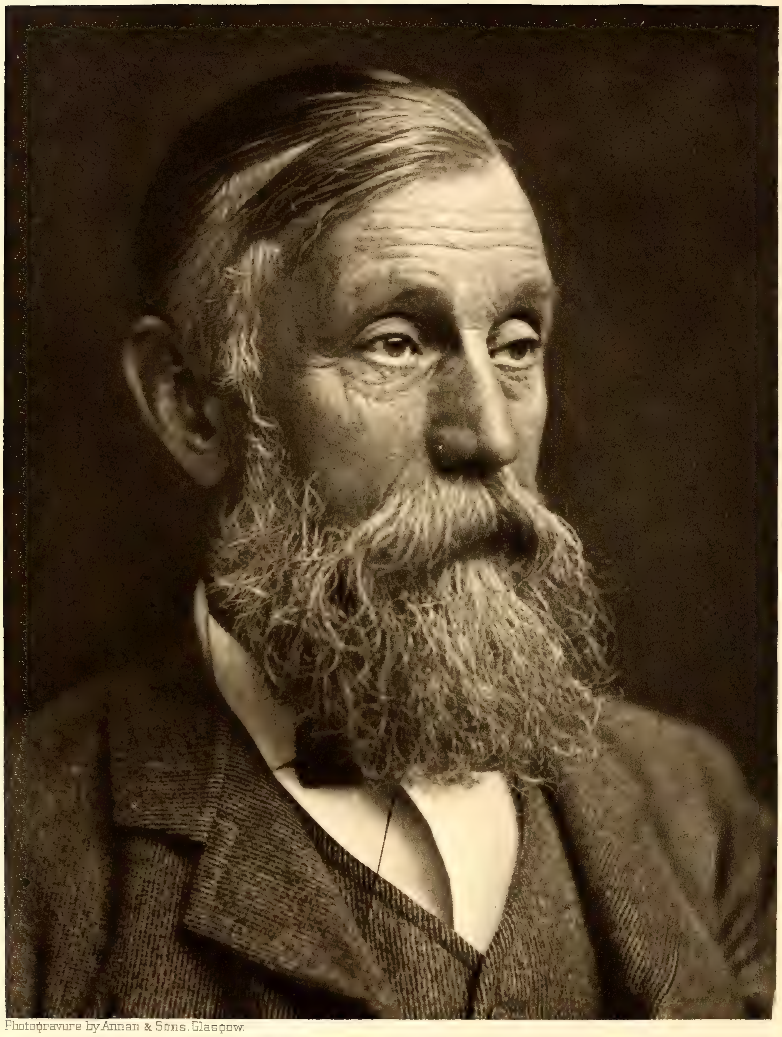

Photogravure by Annan & Sons. Glasgow.

M. Foster

The Natural Mechanism for evoking the Chemical Secretion of the Stomach (Preliminary Communication).

By J. S. Edkins and M. Tweedy.

(Communicated by Professor E. H. Starling, F.R.S. Received July 10,—
Read November 12, 1908.)

The enquiry was directed to answering the following questions:—

1. What is the comparative value of different food substances in evoking the chemical secretion of the stomach ?

2. What channels of absorption are available for the different food substances ?

Experiments in a previous communication to the Society* indicated that the mucous membrane of the pyloric end of the stomach contained the gastric hormone, and was therefore probably a channel of absorption. No direct proof was at that time able to be adduced.

By a method which we have elsewhere described, we were able, for the purpose of the present experiments, to separate functionally the fundus from the pyloric region of the stomach.

Our results indicate that there is practically no absorption in the fundus end of the stomach. It was shown in the previous communication that the mucous membrane at this end did not contain the gastric hormone. At the pyloric end various substances introduced and confined to that region evoke a marked secretion from the fundus mucous membrane. Acid alone is but a slight stimulus. Dextrin has a marked effect. Dextrose and maltose have an effect approximately equal to dextrin. Witte's peptone gives a more pronounced result than the carbohydrates. The meat extract devised by the late Professor Herzen, of Geneva, was found more potent than anything else tried. These different substances introduced into the fundus end, and confined to that end, evoked no secretion. Introduced into the duodenum, they also evoke a secretion, Herzen's extract being most effective.

There was no evidence of any substance passing into the circulation tending to inhibit the gastric secretion. It is shown, therefore, that the pyloric end of the stomach and the duodenum are to be regarded as the normal channels whereby such absorption occurs as liberates the gastric hormone. The fundus end of the stomach is definitely excluded from this function.

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* 'Roy. Soc. Proc.,' B, vol. 76, 1905.

*Further Observations on Welwitschia.**

By H. H. W. Pearson, Sc.D., F.L.S.

(Communicated by Professor A. C. Seward, F.R.S. Received July 29,—
Read November 12, 1908.)

(Abstract.)

The material which forms the subject of this investigation was collected at Welwitsch and Haikamchab, in Damaraland, in January and February, 1907.

Macrospores and embryo-sacs are frequently present in the pith region of the female cone-axis. This confirms the view, already adopted by most authors, that the ovule of Welwitschia is cauline. Sporogenous cells have not been found in a similar position in the male cone.

It is suggested that the female cone and the male flower are derived by reduction and specialisation from an amphisporangiate strobilus of a type similar to that of Bennettites.

At the end of the free nuclear division the embryo-sac contains about 1024 nuclei which are equivalent in all visible characters. Cleavage of the cytoplasm occurs, resulting in the septation of the whole sac into compartments. Those near the micropylar end contain few nuclei which are functionally sexual; most of those of the lower three-fourths enclose many potentially sexual nuclei. The former send out embryo-sac-tubes into the nucellar cone and into them pass the cytoplasm and free nuclei; all the nuclei in each of the latter fuse so that each compartment becomes a uninucleate cell. The compartments containing the fusion-nuclei form the primary endosperm, whose later growth is distributed over two periods, one before and the second after fertilisation. The endosperm of Gnetum is probably formed in the same way. In respect of the morphological character of the endosperm, Gnetum and Welwitschia are widely separated from Ephedra, in which the endosperm is a prothallus of the normal gymnosperm type. It is suggested that the endosperm of the primitive angiosperms was homologous with that of Welwitschia.

The embryo-sac-tubes meet the pollen-tubes in the lower half of the nucellar cone. Fertilisation occurs within the generative cell.

The generative cell enlarges after leaving the pollen-grain and its nucleus divides. The daughter nuclei are functional gametes.

Several oospores are commonly formed in each nucellus. The cytoplasm of

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* Assisted by a grant from the British Association.

the oospore is mainly, if not entirely, provided by the generative cell. A resting nucleus is formed.

The oospore elongates towards the top of the endosperm. The first nuclear division within it is followed by the formation of a centripetally developed wall which separates the upper "primary suspensor" from a lower terminal cell. From the latter are developed: (*a*) 24 cells which, surrounding the lower part of the primary suspensor, form with it the "secondary suspensor"; (*b*) a terminal group enclosing a presumed embryonic plate of eight cells. The later stages of embryo-development have not been seen; they possibly occur, as in Gnetum, after the seed is detached from the plant.

It is suggested that (1) The Gnetum-Welwitschia alliance has its origin in the same stock as the angiosperms, but separated from the angiosperm line before the carpel became the pollen-receiver; (2) Welwitschia is the most specialised living representative of the race to which it belongs.

On the Presence of Hæm-agglutinins, Hæm-opsonins, and Hæmolysins in the Blood obtained from Infectious and Non-Infectious Diseases in Man. (*Preliminary Report.*)

By Leonard S. Dudgeon, F.R.C.P. Lond.

(Communicated by Prof. T. G. Brodie, F.R.S. Received July 31,—Read November 12, 1908.)

(From the Pathological Laboratories, St. Thomas's Hospital.)

This preliminary report is for the purpose of introducing certain results which have been obtained by allowing normal and immune human serum to act in the presence of normal and immune blood cells.*

These experiments have brought to light many interesting and important points in human hæmatology. The unlimited number of experiments which have been made on immune substances in the blood of the lower animals in comparison to similar investigations in man is most striking. It is necessary to draw special attention to the fact that the work of others has been either briefly referred to or omitted merely because this report is only intended to draw attention to the main facts met with in these investigations, avoiding as far as possible a detailed survey of the subject.

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* The expression "immune cell" and "immune serum" is used to designate the blood-cells and sera taken from cases of any general disease or condition. The term "immunity," *i.e.*, is not confined, in this communication, to the series of phenomena resulting from bacterial infection alone.

Nature of Cases investigated.

Every care has been taken to make the classification of the various diseases which have been investigated accurate. Wherever it is permissible, the various groups and sub-groups are arranged as the result of a combination of pathological, bacteriological, and clinical investigations*:—

Group I.—10 cases of acute pneumonia and acute empyæma.

Group II.—22 cases of tuberculosis (mostly acute pulmonary), and 1 case of leprosy.

Group III.—12 cases of epilepsy.

Group IV.—5 cases of acute peritonitis due to appendicitis.

Group V.—5 cases of infections due to the bacilli of the typhoid and paratyphoid family.

Group VI.—26 cases of anæmia, including 14 of pernicious anæmia, chlorosis, lymphæmia, myelæmia, congenital cholæmia, and examples of anæmia secondary to various well recognised conditions.

Group VII.—7 cases of infections due to the streptococci.

Group VIII.—3 cases of infections due to the Staphylococcus pyogenes aureus.

Group IX.—Miscellaneous cases. Infective endocarditis, purpura, jaundice, chronic lead poisoning, chronic renal disease, coal gas poisoning, acute poisoning of unknown origin, eclampsia.

Hæm-agglutinins.

Technique.—The blood was collected for these experiments in 0·85-per-cent. pure sodium chloride, and 0·85-per-cent. pure sodium citrate in distilled water. The corpuscles were then centrifuged and thoroughly washed free from plasma in sodium chloride. A 5-per-cent. suspension of the washed corpuscles was made in normal saline. The blood was also collected in glass tubes and centrifuged in the course of an hour or so when clotting had completely taken place, as by this means a serum was obtained showing no red tinge.

In experiments on hæm-agglutinins, hæm-opsonins, and hæmo-lysins, the investigations were made immediately the blood was withdrawn.

For the agglutinative tests, one measured volume of the 5-per-cent. solution of *washed* red blood corpuscles was drawn into a capillary tube with an equal volume of blood serum. These were then thoroughly mixed and incubated usually for 1 hour at 37° C. in a horizontal position in an incubator suitable for such purposes. At the expiration of this time, the contents of the capillary tube were blown out on to a slide, a glass cover-slip was placed over the mixture and the specimen was examined microscopically. In many instances, the agglutinative effect was obvious to the naked eye.

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* I am greatly indebted to one of the workers in my laboratories, Mr. H. A. F. Wilson, for much valuable help in obtaining samples of the blood from the cases which I was investigating.

In some cases, incubation of the tubes was allowed to continue for several hours, but no advantage was gained by the extra time, and, in some instances, a hæmolytic action interfered with the results.

Experiments were also made with the centrifuged red cells undiluted with salt solution for the agglutination tests, but the results were unsatisfactory.

A definite suspension of *washed* red cells in salt solution, and a definite volume of the corpuscles and of serum were employed, so as to obtain certain essential advantages:—

(1) By employing the washed red cells, any agglutination which occurs must be due to the interaction of the red cells and of the serum added.

(2) We can add any desired serum and examine its properties, which could not be done with the unwashed red cells.

It was found that even in cases in which the agglutinative effect brought about by mixing equal volumes of serum and red cells was marked, yet if we diluted the serum with normal saline previous to the mixing with the red cells, the effect was considerably diminished, and, according to the amount of dilution, rapidly lost. This confirms the observations made some years ago by Shattock. If, however, we diluted the specific serum with an inactive serum, the results were much more satisfactory than by the method of dilution previously referred to.

Hæm-agglutination.

Shattock,* in 1899, communicated a paper to the Pathological Society of London, on chromocyte clumping in acute pneumonia and certain other diseases. His observations were carried out for the purpose of determining whether the blood serum from patients suffering from acute pneumonia, erysipelas, or acute rheumatism, had any effect upon the rouleaux formation of normal human blood. He found, by adding 1 loop of normal human blood to 1 loop of pneumonic serum, that the chromocytes ran together. On the nodal points the discs were clustered into large knotted masses. Similar observations were made in the blood from the other acute diseases just referred to. It is interesting, however, to note that in all these observations mentioned by Shattock in his communication, which was the first on this subject in human hæmatology, he always employed *normal human blood* and added to it the *immune serum*. The importance of this detail will be referred to later.

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* S. G. Shattock, "Chromocyte Clumping in Acute Pneumonia and certain other Diseases, and the Significance of the Buffy Coat in Shed Blood," ' he Journal of Pathology and Bacteriology,' 1900, vol. 6, No. 3, p. 303.

In 1900, Grünbaum* read a preliminary report which was published in the 'British Medical Journal' on the agglutination of red blood corpuscles. He came to the following conclusion as the result of his preliminary investigations: "While the serum from a case of typhoid would clump the corpuscles in the blood from another disease, it did not clump the corpuscles from the same disease. The same held good for scarlet fever."

It appears from the investigations which I have made that it is extremely common to obtain agglutination by allowing normal serum and immune red cells, or normal red cells and immune serum, to interact. In every instance a control experiment was also made by employing normal serum and normal red cells. Agglutination was not observed when normal serum was added to normal red cells, either of the same individual or from another healthy person, with one exception—a serum from an apparently healthy medical student working in my laboratory caused marked agglutination of my red cells. There was no auto-agglutination observed in this instance, and no other normal serum agglutinated either my red cells or his red cells.

The class of cases in which agglutination is most commonly met with appears to be patients suffering from tuberculosis. In this disease agglutination of human red corpuscles by one of the methods already referred to is a matter of common occurrence. In these cases examples of iso-agglutination have been obtained, and also the still rarer phenomenon of auto-agglutination. This can be very well shown in an experiment which was conducted with the red blood cells and the serum obtained from a very severe case of pulmonary tuberculosis:—

No.	Equal volumes of—	Result.
1	Immune serum (tuberculous) and normal red cells ...	Pronounced agglutination was obtained (iso-agglutination).
2	Immune serum (tuberculous) and immune red cells (tuberculous)	Similar result (auto-agglutination).
3	Immune serum (tuberculous) and immune red cells (epilepsy)	Same result (iso-agglutination).
4	Normal serum and normal red cells	No agglutination.
5	Normal serum and immune red cells (tuberculous) ...	Good agglutination (iso-agglutination).

The blood obtained from a case of chronic pulmonary tuberculosis gave a result which is commonly met with. The immune serum caused a high degree of agglutination when added to normal red cells. There was no auto-agglutination, and normal serum did not react when added to the immune red cells of this case.

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* A. S. F. Grünbaum, 'British Medical Journal,' 1900, vol. 1, p. 1089.

In another example of pulmonary tuberculosis, the immune serum did not react when mixed with normal red cells or with the red cells from the same case, but normal serum produced a high degree of agglutination when mixed with the tuberculous red cells.

The agglutinative properties from seven cases of acute pneumonia and two cases of acute pneumococcic empyæma were subjected to a detailed examination, and, as some of the results were of interest, they may be expressed concisely and briefly:—

The serum from one case of acute pneumonia when added to the red cells from the same case gave no result, but when added to normal red cells a high degree of agglutination occurred. When this pneumococcic serum was diluted with salt solution and added to normal red cells, employing dilutions of 1 : 20 and 1 : 200, no agglutination took place.

Another case of acute pneumonia gave results of similar interest. The blood was obtained from a patient suffering from acute pneumonia just after the crisis. The immune red cells and the immune serum when mixed, failed to react, but when the immune red cells were mixed with normal serum, or when the immune serum was added to normal red cells, a high degree of agglutination resulted. The serum from this case was also mixed with the patient's unwashed red cells; the result was similar to that obtained with the washed red cells. The immune serum from the second case of acute pneumococcic empyæma produced very slight agglutination when mixed with the immune red cells. Normal serum gave a similar result with these red cells, but the immune serum in the presence of normal red cells, and of the red cells from another case of acute pneumococcic empyæma, produced considerable agglutination; in fact, the clumps reached an enormous size, and there were very few free red cells present.

In the blood obtained from a case of long standing epilepsy, a striking instance of auto-agglutination was observed. The patient's serum, when added to his own red cells, caused a high degree of agglutination. The same effect was observed when the immune serum was added to normal red blood corpuscles. In this specimen of blood we have one of the few examples, and yet one of the most striking instances of auto-agglutination.

In another case of long standing epilepsy the immune serum failed to react in the presence of the immune red cells or normal red cells, but when normal serum was mixed with the immune red cells, they collected together into large and tight clumps.

Specific Hæm-agglutinins.

In these experiments, if a serum was found to produce marked agglutination when mixed with certain red blood corpuscles in the manner described, it was tested for the presence of specific agglutinins. A definite measured volume of the immune serum was mixed with a similar volume of a thick deposit of the washed red cells obtained at the end of the last stage of centrifugalisation. These red cells were not suspended in saline, so that the serum could be more intimately mixed with a dense volume of red cells, and would not be diluted with salt solution. The mixture was incubated in sealed capillary tubes for several hours at 37° C. It was then centrifuged at high speed, and the clear serum tested on a 5-per-cent. suspension of washed red cells as before.

The first case in which the specific effect may be referred to is one of great interest. The immune serum from a case of acute pulmonary tuberculosis was found to produce a high degree of agglutination when mixed with the red blood corpuscles obtained from a case of anesthetic leprosy, and with normal red cells, but there was absence of any auto-agglutination. The serum from this case was "saturated" with the red blood corpuscles (leprosy) in the manner referred to in the technique. The mixture, after incubation, was centrifuged, and the clear fluid which separated was added to the leprosy red cells and to normal red cells. No agglutination resulted. When the immune serum was saturated in a similar manner with the normal red cells, the clear fluid obtained did not agglutinate normal red cells, and only slight agglutination occurred when it was added to the leprosy red cells. The serum from a case of chronic surgical tuberculosis which had been found to produce a high degree of agglutination when added to normal red cells and the leprous red cells, although it failed to show any auto-agglutination, was saturated with normal red cells. The clear fluid was then added to normal red cells, but no agglutination resulted. When added, however, to the leprous red cells, distinct agglutination occurred. From these experiments, it would seem that the leprous red cells had the power of removing entirely the agglutinative properties of the serum from the case of acute pulmonary tuberculosis; this serum, as the result of saturation, failing to agglutinate either the leprous red cells or the normal red cells. When, however, the serum from the case of acute pulmonary tuberculosis, and from the case of surgical tuberculosis, was saturated with normal red cells, the result was to abolish the clumping action with the normal red blood corpuscles, but to leave a certain degree of agglutination for the red cells of the leper.

The simple agglutinative properties of the blood from the case of acute pneumonia, on which these saturation experiments were made, have been referred to already in detail. The immune serum, when added to normal red cells, caused a high degree of agglutination. When saturation was completed, it was found that the resulting clear fluid failed to agglutinate normal red cells, but still had the power of agglutinating the red cells from a case of epilepsy, although to a less extent than previous to saturation with normal red cells. Normal serum and the pneumonic red cells, when mixed together, caused a high degree of clumping to occur. The clear fluid which resulted from the saturation of this sample of immune red cells and normal serum would not react with the immune red cells, with which excessive clumping had occurred previous to saturation.

The serum from another case of acute pneumonia, just after the crisis, failed to react with the immune red cells or with normal red cells, but normal serum, when added to these immune red cells, produced a high degree of clumping. The examination of the blood in this instance is, therefore, of considerable interest, because the immune serum had no agglutinative effect on either immune or normal red cells, but normal serum which had no clumping action on normal red cells and on certain other examples of immune red cells, yet produced enormous clumping in the presence of the pneumonic red cells. When normal serum was saturated with these immune red cells, the clear fluid that resulted failed to agglutinate them, but produced some degree of agglutination in the presence of a sample of tuberculous red cells which had given considerable clumping with the normal serum previous to agglutination. When the normal serum and tuberculous red cells were mixed together and saturation was completed, the clear fluid obtained would not agglutinate the pneumococcal red cells referred to above or the tuberculous red cells. The immune serum from a case of severe pernicious anæmia and the same specimen of tuberculous red cells showed considerable clumping when brought into contact. When saturation was completed, the resulting clear fluid failed to agglutinate either the tuberculous red cells or the pneumonic red cells which had run together in large masses in the presence of the serum from this case of pernicious anæmia previous to saturation. The last series of experiments completed in this group of specific agglutinative tests are of importance, because they illustrate the fact that saturation of the pernicious anæmia serum with normal red cells, with which it failed to react previous to saturation, had no power to remove the agglutinative property from this sample of immune serum either for the tuberculous or the pneumonic red cells. In this special instance the agglutination was as well marked after as before saturation.

Bacterial- and Hæm-agglutinins present in the same Serum.

The serum obtained from a case of typhoid infection was found to possess a very high degree of agglutination on normal red cells, but did not possess any auto-agglutinative properties. When the patient's serum was saturated with his own red cells, it was found that it still possessed as high a degree of agglutination on typhoid bacilli as before saturation. The same serum when saturated with normal red cells, on which it had a marked agglutinative action, still possessed the same power of clumping typhoid bacilli as in the previous experiments. A further experiment was performed with the serum from another case of typhoid fever. Saturation of this serum, which had caused marked agglutination when added to typhoid bacilli and normal red blood corpuscles, removed the agglutinative action on the red cells, yet it had no effect on the clumping action of the bacilli.*

The Relationship between Rouleaux Formation and Agglutination of the Red Blood Corpuscles.

In normal blood rouleaux formation is a constant factor. In certain acute and chronic diseases the rouleaux formation is either present to a slight degree or is absent. In those cases in which its absence is noted, it may be found that agglutination of the red cells is present instead. It will be seen, however, from previous statements, that auto-agglutination is of rare occurrence, while iso-agglutination of one type or other is extremely common. It is, therefore, not strictly accurate to state that agglutination of the red blood corpuscles in certain diseases replaces or has replaced the rouleaux formation which was present in that same blood in health, because rouleaux formation, as we see it, is a true auto-effect—the red cells and the plasma from the same case. In agglutination it is generally otherwise, that is to say, the clumping of normal red cells in the presence of immune serum or *vice versâ* is an iso-effect. True agglutination, such as may be seen in the experiments which I have referred to, is never to be found in blood obtained directly from the patient. Agglutination of the rouleaux may take place in pathological blood; in some diseases it is very obvious, but here again the small clumps of rouleaux do not resemble actual agglutination of the red blood corpuscles. In a paper on acute lymphocythæmia, which I published a few years back in the 'Transactions of the Pathological Society of London,' attention was drawn to agglutination of the red cells in the fresh blood examined in the last stages of the disease. This, however, is not an example of true agglutination, but it is clumping of short rouleaux. Shattock noted, as already stated, that if we diluted human serum with saline, the red blood corpuscles did not run into rouleaux when mixed with this diluted serum, as in the case of the pure serum. A similar effect is the rule in the case of agglutination. In this respect, rouleaux formation and hæm-agglutination have points in common. If the blood is examined, *e.g.*, from a case of pulmonary tuberculosis in which the rouleaux formation observed in the fresh film is well-nigh perfect and free from agglutination, when this serum is mixed with normal red cells it gives a striking appearance to the blood. No rouleaux formation can be observed, but the red cells are collected into large and tight clumps, while the shape of the cells is considerably altered from the large red circular disc to small retracted bodies apparently less than half the size of the original cell, highly refractile and resembling an oil droplet. This is probably a physical action and may be dependent upon the salt contents of the serum and cells.

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* In this communication only a few of the experiments on specific and simple hæm-agglutinins have been referred to, as it would be unnecessary repetition to cite them all.

From what has been stated in this communication, and from very numerous unrelated observations, rouleaux formation and haem-agglutination must be regarded as distinct phenomena.

Hæm-opsonins.

Technique.—The white cells for these experiments were obtained by the method which is usually adopted for such investigations on phagocytosis. In every instance normal leucocytes were employed, but in some instances washed immune cells were obtained from various cases, and the results compared with those obtained with the normal cells. It was found, however, that practically the same result occurred whether the immune cell or the normal cell was employed for this special purpose.

A 5-per-cent. suspension of washed red blood corpuscles in normal saline (0·85 per cent.) was used in all these experiments. One volume of normal or immune leucocytes was drawn up into a capillary tube with an equal volume of washed red cells and blood serum. These were carefully mixed, sealed in a glass tube, and incubated in the usual manner. Various times were used for the incubation, varying from 15 minutes to several hours, but there was no advantage, while in many cases a disadvantage occurred in incubating for periods of longer time than half an hour.

In many instances the serum, before it was drawn up into the capillary tube, was diluted to a definite strength either with normal salt solution or with a standard normal serum. At the end of the incubation period the contents of the capillary tube were blown out on to a glass slide, and thin film preparations were made and stained with Leishman's stain. It is most important that the film preparations should be thin and rapidly dried, as otherwise the leucocytes retract owing to imperfect fixation, and the estimation of the phagocytosis becomes impossible.

In some instances, the serum was heated to 55° C. for 20 minutes, and the phagocytic properties of the serum were then compared with those obtained with the unheated.

It will be understood from the remarks which have already been given on this subject of phagocytosis of red blood corpuscles, that numerous methods were employed and comparisons made so as to ascertain the manner in which phagocytosis was most marked. It is unnecessary to refer at great length to the very large number of experiments made, as it was only in a few instances that phagocytosis was pronounced. In quite a number of instances, whether the serum was unheated, or diluted, or heated, or whether normal or immune leucocytes were employed, the degree of phagocytosis was infinitesimal. In every instance 50 or 100 leucocytes were counted, and the number of corpuscles engulfed was carefully noted. In the large majority of cases, out of 50 cells only three or four would be found to be phagocytic. There was only one instance out of the total number investigated in which the phagocytosis of red blood corpuscles was a striking feature. This occurred in a case of jaundice, unfortunately of unknown origin. The immune red cells of the patient in the presence of the unheated undiluted immune serum from the same case and normal leucocytes gave a high degree of phagocytosis: 56 per cent. of the cells were phagocytic, and there were 33 red blood corpuscles in the 50 cells. When normal red cells were used in conjunction with the same serum and normal leucocytes, as in the previous experiment, 76 per cent. of the leucocytes were phagocytic, and 50 of these contained 46 red blood corpuscles.

In both these experiments, the serum, the leucocytes, and the red blood corpuscles were incubated in the capillary tubes in equal volumes for a period of 15 minutes at 37° C. The red cells stained well with eosin, showed no degenerative changes, and none of the leucocytes contained definite "Ghosts." This serum was found to possess a high agglutinative property for normal red cells, but it was not tested on the immune red cells. It was found to be free from any hæmolytic action, either on the patient's red cells or on normal red cells.

As previously mentioned, *these were the only two examples* out of some 250 experiments made in which *over* 20 per cent. of the leucocytes were phagocytic. The other instances in which the phagocytosis was sufficiently pronounced to record were, firstly, a case of chlorosis, in which 20 per cent.

of the leucocytes contained red blood corpuscles, the phagocytic mixture consisting of the immune red cells, normal serum, and normal leucocytes. In the same experiment, conducted with immune serum, only three cells were phagocytic.

The next case was an example of severe anæmia, secondary to hæmorrhage, in which 16 per cent. of the leucocytes were phagocytic. In this experiment, as in the last, the higher degree of phagocytosis occurred by the interaction of normal serum and immune red cells.

In a case of acute peritonitis and appendicitis, 20 per cent. of the leucocytes were phagocytic, but in this experiment the higher degree of phagocytosis occurred by the interaction of immune serum and immune red cells, in conjunction with normal leucocytes.

In all the other experiments it was often found that 5 to 10 per cent. of the leucocytes contained one red blood corpuscle, while in many instances there was no phagocytosis present.

It has been suggested that one of the main causes of anæmia is due to the presence in the patient's serum of a certain substance which acts upon the red blood corpuscles, which in turn became devoured by the leucocytes. There is nothing in these experiments to support this view. In those cases in which the anæmia was most intense, the phagocytosis was present to an infinitesimal degree, while in the cases previously referred to in which the phagocytosis of the red blood corpuscles was such a striking feature, the anæmia was quite of a mild type.

As I have already stated, there was no relation in these experiments between the extent of the agglutinative, hæmolysing, and opsonic properties of the same serum. A very large number of these immune sera had a more or less high degree of agglutinative action, yet the degree of hæmolysis which they were capable of producing was only occasionally striking, and the opsonic property was the least marked. Barratt,* in his experiments, conducted with the blood of the lower animals, has shown that even with unheated immune serum, phagocytosis of red blood cells can occur without the serum having either hæmolytic or agglutinative properties, and he favours the view that the phagocytosis is induced by some body which can be placed amongst the order of opsonins. R. D. Keith,† in a paper also published in the 'Proceedings of the Royal Society,' from work done in the laboratories of the London Hospital, came to the following conclusions as the result of some interesting experiments:—"That the substance which induces phagocytosis is partially destroyed by heat, while the hæmolytic amboceptor is entirely thermo-stable." And secondly: "The hæmolytic amboceptor may be present in considerable amount in a hæmolytic serum without inducing phagocytosis, notwithstanding prolonged contact of the amboceptor with the red blood cells."

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* Barratt, "The Phagocytosis of Red Blood Corpuscles," 'Roy. Soc. Proc.,' 1905, vol. 76, Ser. B, p. 524.

† R. D. Keith, "On the Relationship between Hæmolysis and the Phagocytosis of Red Blood Cells," 'Roy. Soc. Proc.,' 1906, vol. 77, Ser. B, p. 537.

The experiments referred to in this communication entirely agree with the observations of Barratt and Keith, conducted with the blood sera and cells from the lower animals. There was nothing to show that the agglutinative, opsonic, or hæmolysing properties of normal or immune sera on red blood corpuscles have any direct relation to one another.

Hœmo-lysins.

Technique.—A 5-per-cent. suspension of washed red cells in 0·85-per-cent. saline in distilled water was employed for these experiments. Normal red cells were allowed to act in the presence of normal serum and immune serum, immune red cells in normal serum, immune red cells in immune serum, and normal and immune red cells in immune serum, to which a definite volume of native and foreign complement had been added.

The serum to be tested was always carefully noted to be free from any blood tinging.

The mixture of red blood corpuscles, serum, and salt solution was incubated, usually for two hours, immersed in sealed glass tubes in water at 37° C., and placed in an incubator at the same temperature. In some cases the incubation period was considerably extended, but two hours was found to be sufficient. At the end of that time the tubes were put in the ice-safe and examined at the end of about 12 hours. The actual dilutions which were employed were as follows:—The capillary tubes were carefully graduated, and in each tube, in every experiment, the total contents amounted to four volumes, of which one volume of washed red cells was a constant quantity, and the amounts of serum and salt solution were in inverse ratio; the first tube contained one volume of washed red cells and three volumes of serum, without any salt solution, while the tenth tube in each series contained 2·5 volumes of salt solution and 0·005 volume of serum.

It should be noted that the strength of the salt solution employed for these experiments was 0·85 or 0·9 per cent., but it was found in these investigations that certain red cells underwent some degree of hæmolysis when presented to a 0·9-per-cent. salt solution. This is a most important fact, because it shows that physiological salt solution has a power of hæmolysing immune human red cells under certain conditions. This special instance occurred in the case of a girl suffering from acute poisoning of unknown origin. Here, although immune serum failed to hæmolyse the immune red cell, yet physiological salt solution produced slight hæmolysis when presented in the same amount as the serum in the hæmolytic mixture, while, when present greatly in excess of the serum, marked hæmolysis occurred.

In all these experiments it was only occasionally that the hæmolytic action was distinctly shown; in the majority of instances no hæmolysis occurred.

In *Group I*—cases of pneumonia—there were two instances in which hæmolysis occurred. The first example was a case of acute pneumonia, just before the crisis, in which a mixture containing 75 per cent. of auto-immune serum produced complete hæmolysis on auto-immune red cells. The hæmolytic action occurred down to a dilution in which the serum was present to the extent of 25 per cent.

The second example was a case of acute pneumonia, just after the crisis, and in this instance the immune serum hæmolysed normal red cells, but failed to hæmolyse the auto-immune red cells. Well-marked hæmolysis occurred with 75 per cent. of serum. On diminishing the amount of serum, the effect became less until 37·5 per cent. of serum caused only a very slight hæmolytic action.

In *Group II*—cases of tuberculosis—hæmolysis was only occasionally met with. In each instance it was the action of the immune serum obtained from cases of acute pulmonary tuberculosis on normal red cells. In these experiments the hæmolysis was well marked, while auto-hæmolysis did not occur.

In *Group VI*—pernicious anæmia. All the cases referred to under this heading were well-marked examples of the disease. It may be of interest to mention that out of the several cases which were examined, in every instance the serum was either bile-tinged or more frequently of a canary-yellow colour, due to the presence of a pigment which failed to give any spectrum, and which was not bile-pigment. In most of these cases neither auto- nor iso-hæmolysis occurred.

In one case extremely slight hæmolysis occurred by the action of immune serum on normal red cells, while there was no auto-hæmolysis.

In another case there was no hæmolysis during the patient's lifetime, but the serum obtained at the *post-mortem* examination, which contained bile-pigment, was found to have a very strong hæmolytic action on normal red cells; 75 per cent. of serum almost completely hæmolysed the red blood corpuscles, while 12·5 per cent. of serum produced distinct but slight hæmolysis. It was found that in each tube in which hæmolysis occurred, clumps of streptococci were present. This streptococcus, which was obtained in pure culture from the hæmolytic tubes and from the blood at the *post-mortem* examination, was an atypical streptococcus, according to the classification of Andrewes and Horder. The micro-organism itself had also the power of exciting hæmolysis, so that the hæmolytic action which was found to take place in this sample of immune serum was not due to the serum itself, but to the streptococci which it contained in such large numbers.

The third example was a severe case of this disease in which there was no auto-hæmolysis, although the blood was examined on several occasions, and there was no auto-hæmolysis even after the immune serum had been complemented, but a high degree of hæmolysis occurred by allowing the immune serum to act on the red blood corpuscles from a case of acute lobar pneumonia; 75 per cent. of serum produced complete hæmolysis; 62·5 per cent. and 50 per cent. of serum produced incomplete hæmolysis; 37·5 per cent. slight, and 25 per cent. very slight, hæmolysis.

In a case of very severe anæmia, due to hæmorrhage, there was no hæmolysis, but the immune serum had the power to a slight degree of hæmolysing normal red cells. The second case was an example of acute lymphocythæmia in which there was no auto-hæmolysis, but a slight hæmolytic action of the immune serum on normal red cells.

Group VII.—In the streptococcal cases hæmolysis occurred in two instances. The first was a case of streptococcal pyæmia due to an atypical streptococcus, in which the immune serum produced well-marked hæmolysis on normal red cells down to a dilution of 25 per cent. of serum, while there was no auto-hæmolysis. The second case was an example of acute erysipelas due to the *Streptococcus pyogenes,* in which only very slight hæmolysis occurred in the presence of 75 per cent. of serum. Here, again, the interaction was limited to the immune serum and the normal red cells.

Group IX.—In the case of chronic lead poisoning there was no auto-hæmolysis, but the immune serum had a slight but distinct hæmolytic action on normal red cells.

In a case of jaundice, probably carcinomatous, there was distinct auto-hæmolysis with 75 per cent., 62·5 per cent., and 50 per cent. of serum. This case, however, was the only example in the whole series of experiments in which there was an auto-hæmolytic action. If this case proved to be carcinomatous, it would be the only example of this disease in the whole series investigated.

Conclusions.

It will be noticed that a striking feature, with one exception, in all these experiments is the absence of the auto-hæmolytic action and the hæmolysing property of the same serum on normal red blood corpuscles.

Preliminary Note on the Occurrence of a New Variety of Trypanosomiasis on the Island of Zanzibar.

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F.R.S. Edin.

(Communicated by J. Rose Bradford, For. Sec. R.S. Received August 5,—
Read November 12, 1908.)

Neither on Zanzibar nor on the adjacent island of Pemba has the occurrence of trypanosomiasis among animals ever been recorded or suspected. The population of Zanzibar, about 170,000, consists mostly of natives. There is a considerable proportion of Indians, and a very small number of Europeans. Horses are comparatively few in number, but there are many donkeys, mules, cattle and goats. Donkeys and mules are largely obtained from the East; cattle and goats are imported mainly from the mainland of Africa, while horses have been obtained from various countries.

The island itself is of coral formation and has much swampy ground. Apart from mosquitoes, there are very few blood-sucking flies: neither Tsetse flies nor Stomoxys have been found.

On February 9, 1908, Mr. Dubash, an Indian veterinary officer attached to the Sultan's stables, noticed, in a livery stable belonging to an Indian, a horse which showed well-marked swelling of the abdomen and sheath. He at once drew my attention to it, when the animal was made the subject of investigation.

The animal was a bay Arab, entire, aged. It showed evident signs of weakness, while its temperature was 103°·6 F. The front of the chest, forelegs, abdomen, sheath and hind legs, all showed evidence of œdematous swelling. The conjunctivæ and Schneiderian membrane were pale, but no petechiæ were observable. No other horse in the stable showed any evidence of being similarly affected.

Blood was drawn from the ear and also directly from the jugular vein. With this blood, films were prepared for microscopical examination. On examination, the red corpuscles were found to be very markedly vacuolated, and among these were found some very small trypanosomes. Their number was, however, so small that considerable care had to be taken in searching for them, and it may be at once said that at no time were they ever very numerous.

Emaciation, which was already marked in the horse, progressed until death, which occurred on February 18.

Post-mortem Examination.

No petechiæ were observable on the conjunctivæ, nor on the Schneiderian membrane, but both were hyperæmic. On removing the skin from the dependent portion of the thorax and abdomen, yellow solidified œdematous material was found, about an inch in thickness, which extended from the sternum to the hind legs.

The blood was pale in colour, but the coagulation power was little, if at all, diminished.

Muscles.—These were pale in colour and somewhat atrophied.

Lungs.—These organs were slightly œdematous and pale in colour; a moderate amount of hypostatic congestion was seen on one side.

Heart.—The contents of the pericardium were not determinable, as the cavity had been accidentally opened during dissection. Both the epi- and the endocardium showed numerous petechial hæmorrhages, varying in size from a pin-point to patches a quarter of an inch in diameter. On the outside they were most numerous near the interventricular groove, and on the inner side at, and around, the attachments of the chordeæ tendineæ.

Liver.—The organ was fairly normal in size, œdematous in consistence, and almost normal in colour.

Spleen.—This organ was only slightly enlarged, pale on section, and of fair consistence.

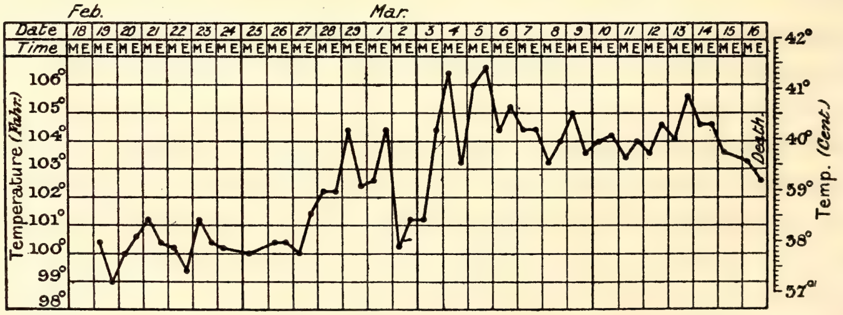
Lymphatic Glands.—These were enlarged and œdematous.

No other organ called for special remark.

Inoculation Experiments.

Owing to the difficulty experienced in obtaining animals, the various animals dealt with were not all inoculated on the same date.

Horse.—A horse was injected intravenously with 5 c.c. of the blood of the infected horse on February 18, 1908. The temperature of the animal



Horse.—Inoculated by intravenous injection of 5 c.c. fresh blood of infected horse on February 18, 1908.

began to rise on the evening of February 27, but already, on February 25 trypanosomes, few but very lively, had been observed in the blood.

On February 28 they were fairly numerous and at their maximum, since from this time up till March 8 they appeared in very few numbers. On March 2 the sheath of the penis and the scrotum were much swollen, and on March 4 the animal was obviously sick, standing with head down and refusing food. By March 7, however, the scrotal swelling was almost entirely reduced; trypanosomes had disappeared from the blood, and the animal looked much better. I left by steamer for England on the morning of March 8, and am indebted for the subsequent history to members of the staff of the Health Department. The improvement noted previous to my departure had been merely transient, since emaciation still progressed with some fever, and the animal died on March 16.

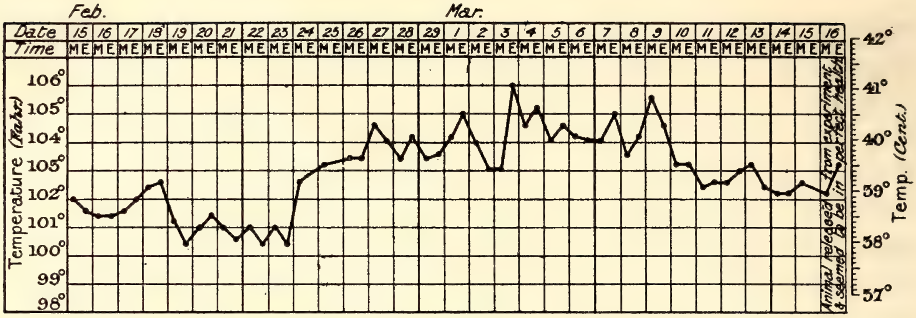
At the *post-mortem* examination solidified yellow œdematous material had been found under the skin. The spleen and liver are said to have been congested.

Donkey.—This animal was injected intravenously with 3 c.c. of the blood of the infected horse on February 14. Within the three days subsequent to this operation, the temperature was above normal, but this is probably to be accounted for by the change of diet and its usual surroundings. From this time onward nothing abnormal was observed, nor was anything of the nature of fever seen. It was discharged from experiment on March 16, when it seemed to be in perfect health. No trypanosomes were seen in its blood at any time.

Dog.—A small pariah dog was inoculated by subcutaneous injection with 3 c.c. of infected blood on February 14. Examinations of its blood were frequently made, but no trypanosomes were seen at any period up till the time of my departure for England, and the animal was discharged from experiment on March 10, when it seemed in perfect health. It has to be noted that the inoculation in this case was by subcutaneous injection, and it is very possible that infection might have been made had some other means of inoculation been used.

Goat.—A goat was inoculated by intravenous injection with 3 c.c. of infected blood of the horse, on February 14. During the first four days the temperature was slightly above the normal, and then subsided. On February 24, however, it began to rise, and attained a maximum on March 3, when it had reached to 106° F.

During the subsequent four days it was steadily falling. At no period were trypanosomes ever found in the blood, nor did the animal at any stage ever show visible signs of any indisposition.



Goat.—Inoculated by intravenous injection of 3 c.c. of fresh blood of infected horse on February 14, 1908.

Ox.—A young ox was inoculated by intravenous injection with 3 c.c. of the blood of the infected horse on February 14. The temperature of the animal was never elevated. From February 20 until the 22nd of the same month it underwent a descent, reaching to 99°, but had again regained the usual mean, about 102°, on February 25. Trypanosomes appeared in the blood on February 27, were fairly numerous by March 2, after which they became few in numbers. The animal never showed the slightest sign of illness.

Monkey.—A small grey monkey was inoculated by subcutaneous injection on March 2 with 2 c.c. of the blood of the ox which, at this time, showed numerous trypanosomes. The monkey was then taken on board the ship with me and seemed in fairly good health throughout the voyage. Nothing was observed in any way abnormal until the morning of May 17, when it suddenly became affected with abdominal pain and passed into violent tremors. Convulsive seizures were then observed, with biting movements somewhat epileptiform in character, and within a couple of hours the animal died. On making *post-mortem* section the blood was found to be somewhat thin in character. There was some acute peritonitis. The spleen was pale in colour and slightly enlarged while the lower end was dark in colour. This dark portion was clearly delimited from the rest of the organ. No other abnormal condition was observed. On examination of the blood, chains of streptococci were observed but not very numerously. No trypanosomes were ever observed in this animal.

Guinea-pigs.—One was inoculated by subcutaneous injection on February 10 with about 1 c.c. of the blood of the infected horse and again on March 2 with 2 c.c. of the blood of the ox, which at this time showed numerous trypanosomes.

One was injected intraperitoneally on February 18 with 1 c.c. of the blood of the infected horse and the operation was repeated on March 2, using 2 c.c. of the blood of the ox. Trypanosomes were not observed at any period up to March 8. The former animal died at sea during a heavy gale, while the other was sent to the Brown Institute after my arrival in London on April 6.

Rabbits.—One was injected intraperitoneally with 2 c.c. of the blood of the ox on March 2 while the other was injected, at the same time, with 2 c.c. of the same blood, subcutaneously.

The subcutaneously injected rabbit was despatched from the port of Marseilles to M. Mesnil, at the Pasteur Institute in Paris, and I have had the pleasure of several reports from him on the matter. It would seem that no trypanosomes have ever appeared in that animal, which remains still in good health. The intraperitoneally injected animal was taken by me to the Brown Institute and there I found several trypanosomes in the blood. They were, however, extremely few and appeared very sluggish as compared with those seen in the horses and the ox.

Rats.—Two large grey rats were fed with infected horse blood on February 14 but never seemed to have become infected.

Description of the Trypanosome.

Its length as seen in the blood of the horses appeared to be from 13 μ to 16 μ, the mean being 15 μ. The breadth measured from 0·75 μ to 1·25 μ, the mean being 1 μ. The blunt end is sometimes rounded but in many it tapers to a sharp point.

The nucleus is placed about 4 μ from the blunt end and measures from 2 μ to 3 μ in length, being most usually about 2 μ. The breadth approximates to 1 μ but extremes may be found from 0·75 μ to 1·25 μ.

The thickness of the parasite is usually greatest between the centrosome and the nucleus. The centrosome, which rarely showed evidence of division, is placed close to the blunt extremity; sometimes a space about 1 μ in extent intervenes between it and the actual extremity.

In size, this trypanosome somewhat resembles the *T. dimorphon*, but is smaller and rather more delicate. M. Mesnil, who has kindly given me his opinion on the parasite, confirms me in this view, and states that he also considers it as being different from the *T. congolense*, which latter M. Laveran considers as distinct from the *T. dimorphon.*

On my arrival in London, the infected guinea-pig, rabbit, and monkey were placed under the charge of Professor Rose Bradford, who kindly arranged to have further investigation made of this interesting parasite. I take this opportunity of expressing my thanks to him for his kind assistance.

On Methods for the Continuous (Photographic) and the Quasi-Continuous Registration of the Diurnal Curve of the Temperature of the Animal Body.

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(Received June 15,—Read June 18, 1908.)

(Abstract.)

In this paper, the author, after referring at some length to the earlier stages of the investigations of the temperature of man, and pointing out that the first approximate attempt to study the diurnal curve of the temperature of man commenced with the investigations of Jürgensen, of Kiel, whilst the systematic study of temperature in disease dates from the time of the researches and publications of Wunderlich, discusses the methods which he has adopted: *(a)* for the absolutely continuous and *(b)* for the quasi-continuous, registration of the diurnal curve of the temperature of man.

After referring to the method employed by Benedict and Snell for the quasi-continuous record of the curve of temperature, and which, as he points out, is in no sense an automatic method and involves, in a very serious manner, the errors due to the "constantly varying personal equation" of the observer, he declares himself a strong partisan of a thermo-electric method of recording exceedingly minute variations of temperature. Since the time when, early in life, he studied the question, the experimental conditions have changed in certain very important particulars:—

1. By the discovery of galvanometers of the moving-coil type which we associate with the name of D'Arsonval, but which had their prototype in the syphon-recorder of Lord Kelvin, and which are practically uninfluenced by changes in the surrounding magnetic field.

2. By the remarkable improvements in apparatus for maintaining a practically constant temperature in considerable masses of liquids, so that a thermocouple may be maintained during days and weeks at so constant a temperature as to admit of the variations being absolutely neglected.

3. The employment of photographic recorders which admit of the registration of the movements of mirrors, as in magnetographs and seismographs has, further, facilitated such investigations as the one under discussion.

Under the headings (1) The Thermostat and its Regulators; (2) The

Thermo-couples, *i.e.*, the electric thermometers employed, and the leads; (3) The Automatic Photographic Recorder, the author develops his method of continuous photographic registration, and he dwells particularly on the perfection of the arrangement of the thermostat which he has, with the help of Herr Fritz Koehler, the University Mechanician, of Leipzig, devised. He further discusses, at some length, the arrangements of the photographic recorder constructed for him by the Cambridge Scientific Instrument Company, dwelling particularly on the optical arrangements which have been adopted, and which have resulted in an extraordinary clearness and sharpness of the curves furnished by the instrument.

Discussing, in the next place, the method of quasi-continuous registration of the curve of the temperature of man, in which an ink-record is inscribed on a comparatively slowly rotating cylinder, the author describes "the thread recorder" of Mr. Horace Darwin, F.R.S., specially wound, and with a more than usually delicate suspension, as the special instrument for the record of the curve of the temperature of man for such clinical investigations as will be carried out in future in hospitals and sanatoria.

The paper, which is one concerning methods of investigation rather than results, closes with some illustrations exhibiting the application both of the continuous and the quasi-continuous methods to the study of the temperature of the animal body.

The result which comes out very strikingly, and for the first time, from these first applications of very exact and automatic methods to the study of the heat changes in the animal body, is that the temperature in any given area never remains constant during two consecutive minutes or fractions of a minute, in consequence, no doubt, of varying, and in some cases, perhaps, rhythmical, variations in the action of the great vaso-motor centre and the centres which are subordinated to or in relation with it.

On Reciprocal Innervation of Antagonistic Muscles. Twelfth Note.—Proprioceptive Reflexes.

By C. S. Sherrington, D.Sc., F.R.S.

(Received August 10,—Read December 10, 1908.)

(From the Physiology Laboratory, University of Liverpool.)

The following two reactions, observable in the extensor muscle of the knee, appear not without importance for the reflex co-ordination of antagonistic movements at that joint. They are reactions favourably studied in decerebrate rigidity. The cat is the animal in which, under that condition, my results have been chiefly obtained.

The reactions can be observed as follows:—In the decerebrate animal a preparation of the extensor of the knee is so made that by detachment of other muscles, or severance of other nerves, the vasto-crureus muscle, with its nerve-branch from the anterior crural trunk, remains the sole nerve-muscle component intact in the whole limb. The vasto-crureus is one of those muscles which, after decerebration, exhibits the marked tonus characteristic of decerebrate rigidity. This tonus of the vasto-crureus then maintains the knee in an attitude of partial or complete extension. The flexor muscles of the knee, together with all the other muscles acting on that joint, have been paralysed by section of their nerves.

I. If the nerve of one of the flexors, *e.g.*, of semitendinosus, be now faradised distal to its place of severance, the stimulation of the motor fibres in it causes contraction of the flexor muscle, the knee is consequently flexed; the flexor muscle, by its stronger contraction, bends the knee in spite of the tonic contraction going on in vasto-crureus, the extensor muscle. On discontinuing the faradic stimulation of the motor nerve of the flexor, the flexor muscle, of course, ceases to contract. It is then seen that, although the flexor is no longer acting, the knee still continues to remain flexed. In other words, the tonic contraction of the extensor of the knee, which prior to the contraction of the flexor kept the knee extended, ceases to do so after the movement of knee-flexion has been executed. Evidently the forced stretch given to the knee-extensor by the contraction of its antagonistic muscle has produced some change in the tonic condition of the knee-extensor. It has been previously shown* that centripetal impulses from the knee-flexor can cause a reflex inhibition of the knee-extensor. But in the experiment now described no centripetal impulse from the knee-flexor can have caused the effect, because the nerve of that muscle had been severed above its place of stimulation; indeed, all the nerves of the limb had been severed except only that of the vasto-crureus (extensor muscle) itself.

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* Sherrington, 'Roy. Soc. Proc.,' vol. 52.

The result, therefore, if traceable to centripetal impulses, is traceable to some among them which have their origin in vasto-crureus itself. In accord with this one finds (fig. 1, *L.r.*[1]) that on simply taking with the hand the desensitised limb below the knee, and gently but firmly flexing the tonically extended knee, the same result is obtained as that just described when the knee is bent by the flexor muscle. The forced movement, as executed by manipulation, experiences resistance from the tonic contraction of the extensor (vasto-crureus), but as the flexion movement is executed this resistance somewhat abruptly lessens almost to vanishing; on then discontinuing the forced flexion the limb is found to remain practically where the forced movement (figs. 2A, 4A, *L.r.*) had carried it. In short, the extensor muscle, in result of a forced stretch, assumes in permanence a new tonic length. This reaction of the extensor may, for brevity, be termed the "lengthening reaction."

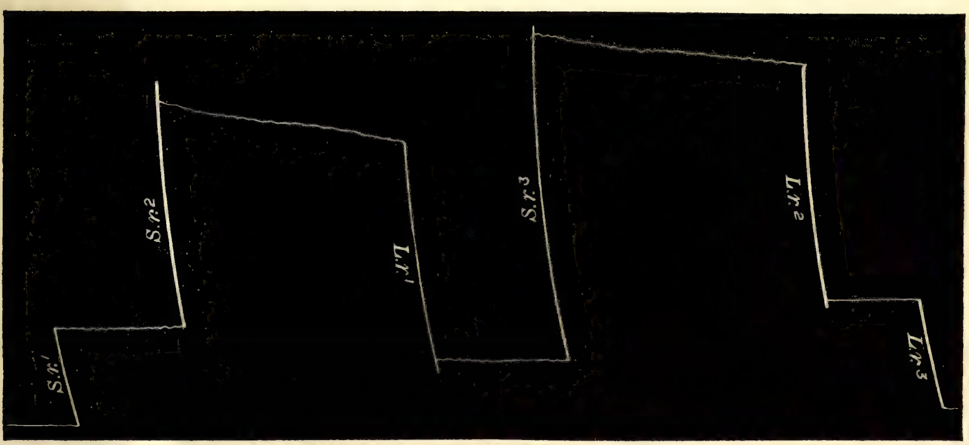

Fig. 1.

The tonus exhibited by the extensor in decerebrate rigidity has been shown to be reflex in nature,* and to depend on afferent nerve-fibres arising in the extensor muscle itself.† The lasting increase in the tonic length of the muscle brought about by stretching it appears to be likewise reflexly produced. Evidence indicating this is as follows:—(1) The lengthening reaction is not obtained after severance of those afferent spinal roots through which pass the afferent nerve-fibres proceeding from the muscle (vasto-crureus) itself. (2) Together with the "lengthening reaction" of the extensor, itself stretched, there is excited by the stretch a contraction in the extensor of the crossed knee. (3) There exist in the nerve of vasto-crureus afferent fibres which, under mechanical and electrical stimulation, cause relaxation of the vasto-crureus muscle itself.* (4) The increase of tonic length of the vasto-crureus muscle induced by the stretch is sometimes, under conditions favourable to reflex rebound† (successive spinal induction), followed by increased contraction of the muscle, just as is reflex inhibitory relaxation caused by afferent nerves known to act inhibitorily upon it. (5) Strychnine annuls the lengthening

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* Sherrington, 'Journ. of Physiology,' vol. 22, 1898.

† *Ibid.*

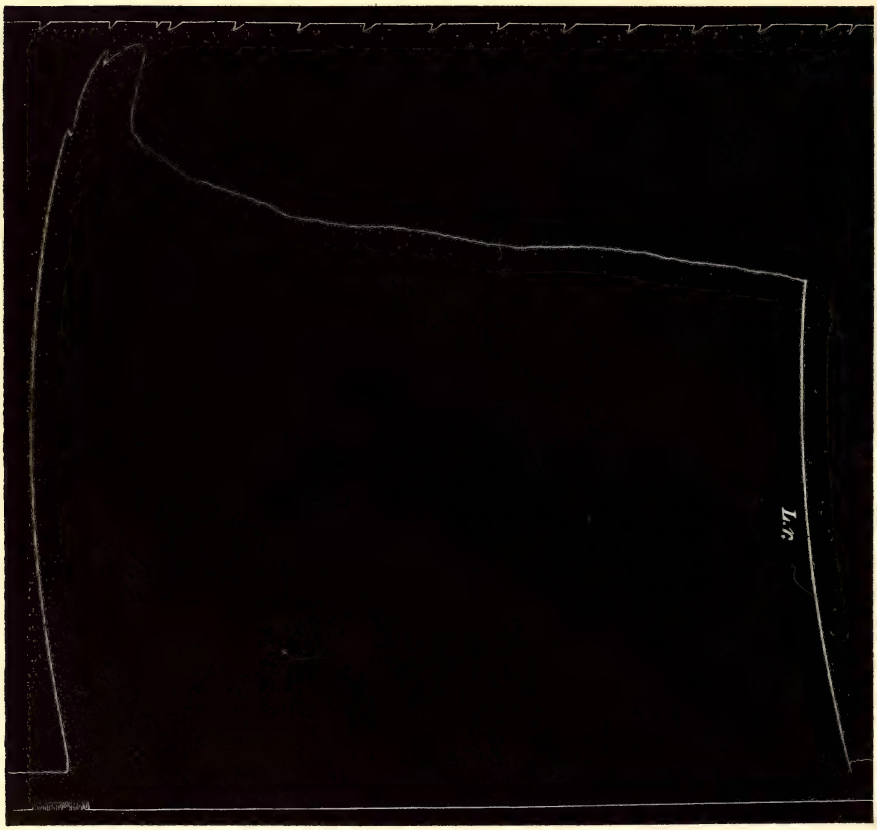

Fig. 2A.

Fig. 2B.

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* Sherrington, 'Roy. Soc. Proc.,' B, vol. 77, 1906.

† Sherrington, 'Roy. Soc. Proc.,' B, vols. 76 and 77; 'Journ. of Physiology,' vol. 36, p. 185.

reaction of vasto-crureus, as it annuls likewise other inhibitory relaxations of that muscle known to be reflex in nature.*

It appears, therefore, that the "lengthening reaction" of the extensor of the knee is a reaction excited by forced stretch of the muscle acting on the reflex tonus through receptive nerves arising in the muscle itself.

II. There is similarly observable in the decerebrate extensor muscle another reaction practically the converse of the foregoing. Suppose that in the limb of the decerebrate animal, prepared as above, the knee, in consequence of the "lengthening reaction" just described, is remaining flexed. Suppose, then, some skin-surface or afferent nerve appropriate for evoking reflex extension of the knee is stimulated either mechanically or by faradisation. The vasto-crureus, isolated as above, contracts, and in consequence the knee is extended. After cessation of the application (figs. 2 and 3, A) of the stimulus, the knee, instead of falling back into flexion, remains extended, and may remain so for many minutes. Light is again thrown on the nature of this persistence of the extensor effect by comparing with it the effect of passive manipulation of the limb. Suppose that, as before, the limb starts with its knee flexed. Then if the observer simply take the desensitised limb below the knee, and move it with his hand into a posture of extension at knee, on his releasing the limb the knee does not drop back into the flexed posture; it retains almost to the full the new extended posture given it by the manipulation (fig. 1, *S.r.*). Passive approximation of the ends of insertion and origin of the vasto-crureus has caused that muscle to assume a new tonic length, shorter than it exhibited before.

This same lasting change in the tonic length of the muscle results whether the initiatory approximation of the ends of the muscle be made by passive manipulation or by active contraction of the muscle itself. The maintenance of the extended knee-posture which follows a reflex contraction of vasto-crureus presents the same characters as the maintenance of posture following a simple passive lift of the knee. If during the application of the exciting stimulus, and for a second or two longer, the knee which the reflex would extend be mechanically prevented from extending, it does not on release assume the extended posture. Prevention of the shortening of the muscle in the first stage of the reflex prevents the occurrence of the long-maintained extension after-effect of the reflex, although that usually endures for minutes.

The conclusion indicated is, therefore, that the shortening of the muscle, produced either actively or passively, induces it to assume in relative permanence a new tonic length shorter than that which it had previously. This reaction may for brevity be termed the "shortening reaction."

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* Sherrington, 'Roy. Soc. Proc.,' B, vol. 76, 1905.

This reaction appears on grounds very similar to those advanced in regard to the "lengthening reaction" to be reflex, and to be traceable to reflex arcs arising in the muscle itself. (1) The "shortening reaction" of vasto-crureus is not obtained after severance of those afferent spinal roots through which pass the afferent nerve-fibres proceeding from the vasto-crureus muscle.

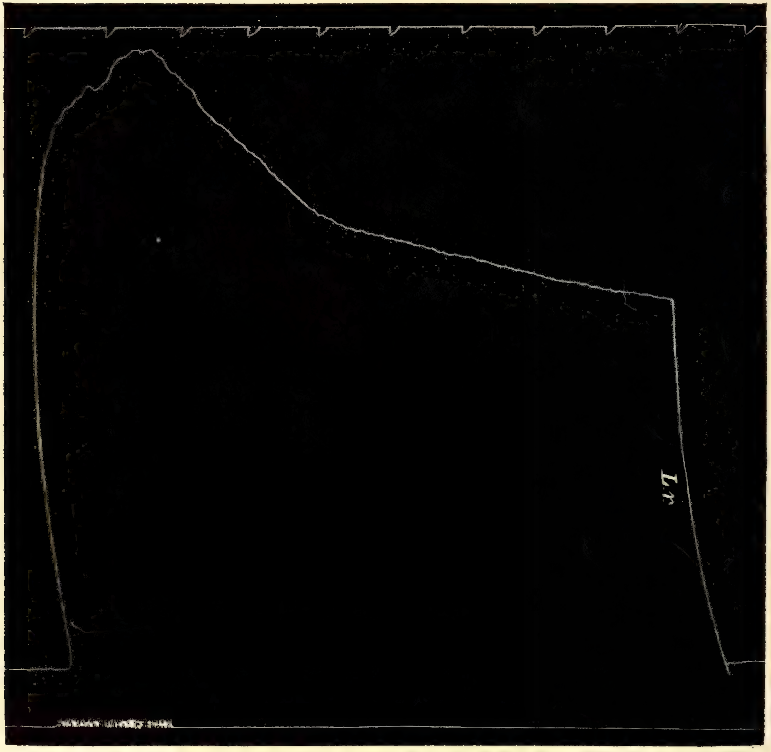

Fig. 3A.

Passive manipulation then fails altogether to elicit it. Where the afferent spinal roots of one vasto-crureus had been cut 20 to 80 days prior to comparing the reflexes from the two vasto-crurei in the decerebrate animal, the reflex contractions exhibited by the vasto-crureus with afferent fibres cut (fig. 2B, fig. 3B, fig. 4B) showed nothing of the long maintained tonic after-contraction exhibited by the fellow muscle whose afferent fibres still remained intact (fig. 2A, fig. 3A, fig. 4A). Reflex contraction of the muscle with its afferent nerve-fibres cut is, therefore, when evoked by slowly repeated stimuli, more clonic than is that of the muscle with its afferents intact (fig. 5, A and B).

(2) Among the afferent nerve-fibres proceeding from vasto-crureus are some which can evoke and maintain reflex tonic contraction of the muscle itself. That seems proved by the dependence of the decerebrate rigidity of this muscle upon the afferent fibres of the vasto-crureus nerve.* (3) When the "shortening reaction" is elicited in the decerebrate animal it is in some

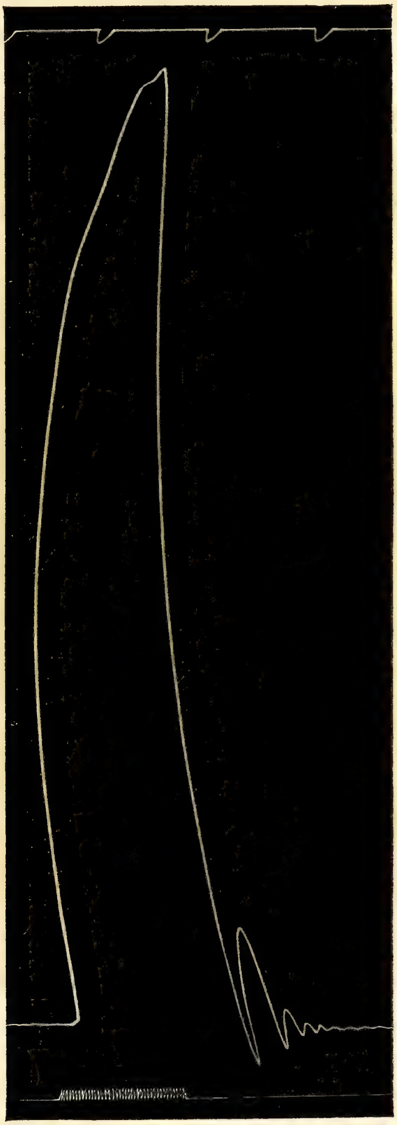

Fig. 3B.

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* Sherrington, 'Journ. of Physiology,' vol. 22.

experiments accompanied regularly by inhibition of reflex contraction of the crossed vasto-crureus. This can be well seen in the double vasto-crureus preparation, the animal being inverted and the extension of knee maintained by vasto-crureus against the weight of the limb below the joint; the occurrence of the "shortening reaction" in one vasto-crureus is then followed by the dropping of the opposite knee into flexion. This crossed inhibition, which obviously must be reflex, accompanies the elicitation of the "shortening reaction."

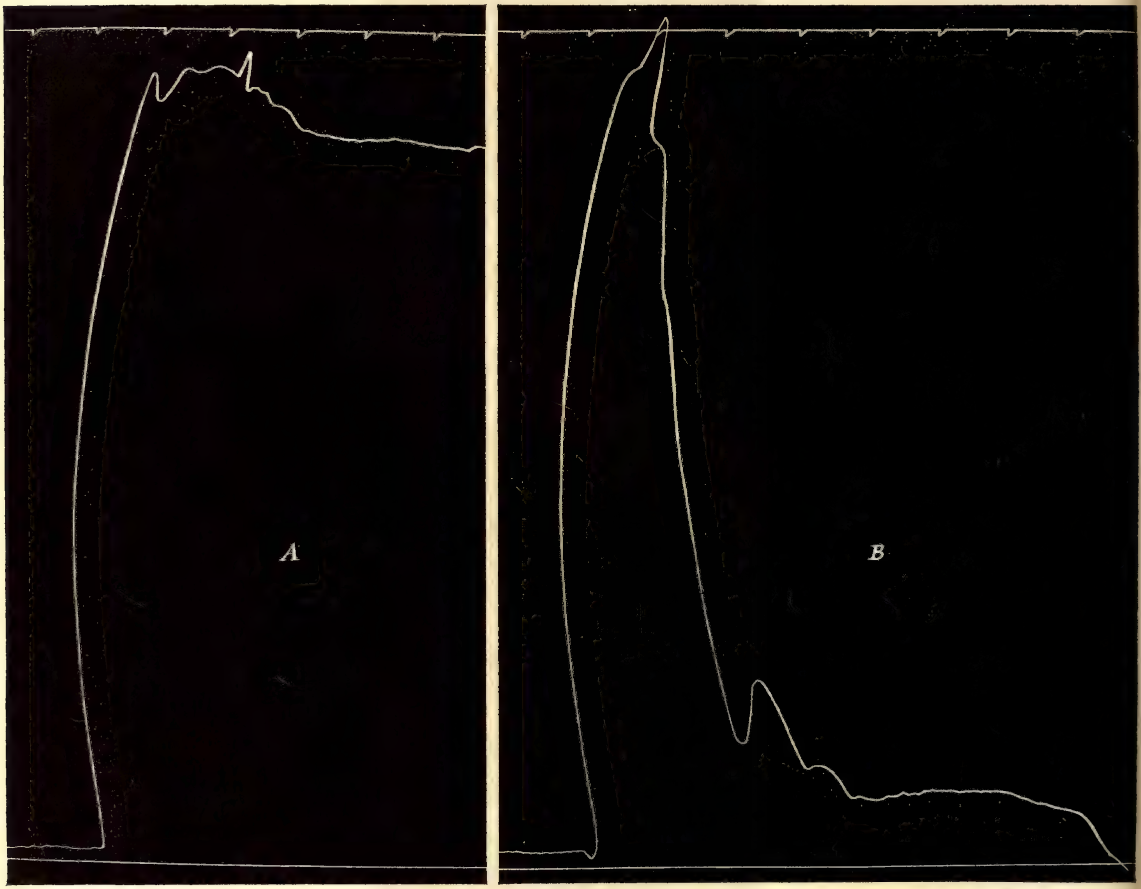

Fig. 4.

The evidence seems to warrant the conclusion that in the rigidity of the decerebrate vasto-crureus muscle the tonus of the reflex arc which maintains the rigidity of the muscle is regulable in two opposite directions by reflex influences which can be started from the muscle itself. Regulation in one direction is shown by the "lengthening reaction," which is a reflex excited by

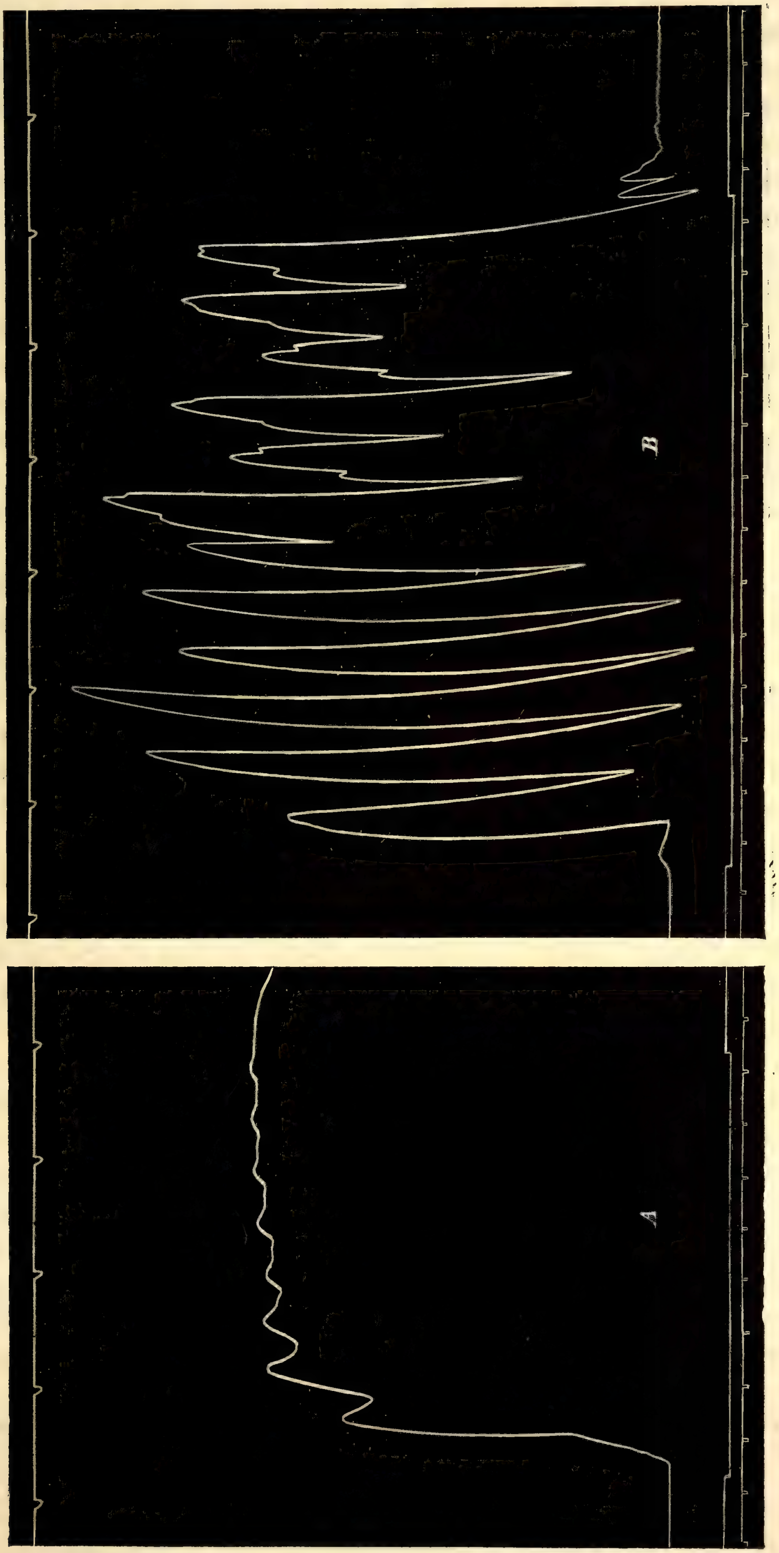

Fig. 5.

stretching the muscle; regulation in the other direction is shown by the "shortening reaction" which is a reflex initiated by approximating either passively or actively the ends of the muscle. By these means there is conferred upon the tonus of the extensor muscle a quality of plasticity. This plasticity enables the tonus to serve as an adjuvant to the reflex contraction of the muscle and also to assist the control of the muscle by its antagonist in the play of opposed movements succeeding each other at the joint upon which both muscles act.

III. The "lengthening reaction" and "shortening reaction" are readily studied in the vasto-crureus preparation above mentioned, but they are also well observable in other muscles exhibiting the rigidity of the decerebrate condition. If the preparation of the hind limb be modified so as to yield a gastrocnemius or soleus preparation, both reactions are evident in those extensors of the ankle. The preparation affords opportunity for determining a point less easily resolved by the vasto-crureus preparation. The nerve of vasto-crureus is credited with supplying a twig to the knee-joint and the question arises whether afferent nerve-fibres from the joint are not responsible for initiating the reactions described. To answer this, the isolation of the vasto-crureus from the knee-joint is required. When this is done, I find the reactions still obtainable. The afferents from the joint are therefore not essential for the reactions; they may, however, be contributory to them. In the case of the nerves to gastrocnemius and soleus, these do not supply twigs to either knee or ankle joint. The obtaining of the lengthening and shortening reactions in the gastrocnemius-soleus preparation shows, therefore, that in this case articular afferent nerves are not even contributory to them.

The "lengthening" and "shortening" reactions are also observable in the extensor muscles of both the shoulder and elbow; probably they are so also in the extensors of the neck and tail and the retractors of the head.

IV. These reactions observable in decerebrate rigidity are also obtainable after spinal transection in the dog. The extensor muscle of the knee exhibits considerable tonus in the dog after spinal transection in the thoracic region when time is allowed for subsidence of the spinal shock immediately consequent on the operation. In the knee-extensor of the spinal dog the "lengthening reaction" is easily obtained, and it is then more regularly than in the decerebrate cat accompanied by crossed extension at the opposite knee. The crossed extension of the opposite knee induced by passive flexion of the other knee has been observed by Philippson* in his spinal dogs and has been termed by him "crossed reflex III." The "shortening reaction" in the knee-extensor of the spinal dog is in my experience also readily evoked. To evoke it, the animal may be held up under the shoulders with the hind limbs hanging freely and the knee then gently raised by lifting it from under the lower end of that joint. This somewhat flexes the hip and extends the knee. A strong tonic contraction of the knee-extensor at once sets in and is usually long maintained. This reaction is frequently though not regularly accompanied by a movement of flexion at the crossed knee.

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* 'Travaux du Laboratoire de Physiologie, Institut Solvay, Bruxelles,' published by P. Heger, vol. 7, part 2, p. 31, 1905.

There is another crossed reflex, well seen in the spinal dog, which bears resemblance to the two just mentioned. A movement of extension of one hip causes a movement of flexion at the crossed hip. To evoke this all that is necessary when the dog is supported as described above is to allow one of the thighs to drop into extension. The opposite hip flexes almost immediately the extension of the other commences.

V. The reflex tonus of these skeletal muscles of the cat and dog is thus seen in these observations to be adjusted by a regulation which conforms, at least in regard to the results it ensures, with features observed by v. Uexküll in the tonus of the musculature of Echinus, Ophioglypha, and Sipunculus. v. Uexküll showed that in those cases stretching the muscle lessens the tonus in it. "Stretching brings about a fall in the tonus of the muscle; the stretched muscle has a lower level of tonus than the unstretched.* When an unstriped muscle is stretched it follows the stretch readily. When the stretch is removed the muscle does not quickly return to its previous length. It exists, therefore, after removal of the stretch, in a different state of excitation from before the stretch. For although it once more is loaded only with its own weight, it is nevertheless longer than before."† v. Uexküll assumes, if I follow him rightly, that a shortened condition of the muscle evidences greater tonus, a lengthened condition less tonus. That may be so, but at present I hesitate to assume it. Apart from this, which seems a difference rather of interpretation than of fact, this description quoted here from v. Uexküll applies fully to the case of the vasto-crureus in decerebrate rigidity. And v. Uexküll's observations on this feature of the tonus of muscles in Echinus, etc., have been found by other experimenters to hold good in the unstriped muscles of various other Invertebrata and in the unstriped muscles of various viscera in Vertebrates.‡ With this feature of behaviour of the tonus in unstriped muscle that of the tonus of the extensor muscles in the spinal dog and in the decerebrate cat conforms.

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* 'Ergebnisse d. Physiol.,' part 2, 1904, and 'Zeitschr. f. Biolog.,' vols. 44 and 46.

† *Ibid.*

‡ *Cf.* Grützner, 'Ergebn. d. Physiol.,' part 2, 1904.

VI. These reactions and the other accessory crossed ones dealt with above belong, evidently by the mode of their elicitation, to that class of reflexes which have been termed *proprioceptive.* By proprioceptive reflexes are meant reflex reactions excited habitually by the organism acting as agent upon itself through its own organs applied as stimuli to its own nerves. In proprioceptive reflexes the organism applies itself as a stimulus to itself. By its own act, and in its own substance, it excites one or more of its own receptor organs. Other reflexes—and they form the major part of reflex action—are excited by environmental change acting through some environmental medium which impinges directly on the receptors of the organism. But the case of, for instance, the bending of the knee, regarded as an exciting stimulus, is different from those. In it the organism, by executing a movement of a part of itself, supplies by the alteration of the condition of that part a stimulus to certain reflex arcs, proprioceptive arcs arising in that part. The reaction thus excited from these arcs is causally related less directly to the environment than are reflexes excited immediately by the surrounding world; the proprioceptive reaction stands in secondary relation to an environmental cause. It is obvious that proprioceptive reflexes may be competent to extend and complete reflex actions, and cycles of reflex action, initiated primarily by environmental stimuli. They thus prolong, by intrinsic proprioceptive action, actions initiated by environmental stimuli of even transient operation.

It was pointed out elsewhere* that proprioceptive reflexes tend to be characterised by certain features: (1) to be tonic; (2) to ally themselves with, and reinforce other reflexes, exteroceptive and interoceptive; (3) to restore a posture which a phasic reflex has disturbed, and thus to contribute toward reinstating a previous reflex equilibrium which had been departed from, *i.e.*, to be compensatory. The reactions described in this note bear out and illustrate these proprioceptive functions. They are tonic. They tend to ally themselves to other reflexes. Taking the case of the extensor of the knee, suppose some environmental stimulus, impinging on the standing animal, impels it to take a step. The flexor of the knee, thrown into contraction, bends the knee; by so doing it stretches the knee-extensor and excites from it a proprioceptive reflex of relaxation (the "lengthening reaction"). This proprioceptive reflex of the knee-extensor is adjuvant to the contraction of the knee-flexor. Then, the phase of knee-flexion having passed, the phase of knee-extension sets in (induced perhaps by successive spinal induction); the knee-extensor contracts and the knee-flexor ceases to contract. The contraction of the knee-extensor shortens the extensor muscle, and by so doing excites in it a proprioceptive reflex (the "shortening reaction"), reinforcing and subsequently maintaining its own contraction. The step has been taken, and both to the taking of it and to the resumption of it anew and to the ensuing maintenance of the standing posture, important contribution has been made by proprioceptive reflexes of the extensor muscle itself.

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* Sherrington, 'Integrative Action of the Nervous System,' London, 1906, chaps. 4 and 9.

EXPLANATION OF FIGURES.

FIG. 1.—Vasto-crureus preparation; decerebrate cat. The base line shows the position of the leg when the knee is flexed. The first rise at *S.r.*[1] is due to lifting the leg below the knee into a less flexed position, and the horizontal line running to the right from that point shows the maintenance of the new posture by the tonus of vasto-crureus. The second ascent at *S.r.*[2] is due to a second passive lift of the leg below the knee; the horizontal line running to the right shows the increased degree of extension of the knee now maintained by the tonus of vasto-crureus. At *L.r.*[1] the knee was passively flexed nearly as far as the original degree of flexion, the horizontal line running to the right shows that the knee is now again maintained by the vasto-crureus in the new posture of flexion. The ascent of the line at *S.r.*[3] is again due to a passive extension of the knee; the horizontal line running to the right from near the top of the ascent shows the maintenance of the new extended posture of knee by the tonus of vasto-crureus. The descent *L.r.*[2] is due to a forced flexion of knee, and the horizontal line from its base indicates the maintenance of the new position of flexion by the tonus of vasto-crureus once more. The descent *L.r.*[3] marks a further forced flexion of the knee, bringing the knee to about the same degree of flexion as that from which it originally started; the final horizontal line shows how the tonus of vasto-crureus maintains the limb once more at this level. *S.r.*[1], *S.r.*[2], *S.r.*[3]: "shortening reactions"; *L.r.*[1], *L.r.*[2], *L.r.*[3]: "lengthening reactions." During the execution of each passive movement the recording surface was not allowed to travel.

FIG. 2.—Crossed reflexes produced in vasto-crureus by faradic excitation of the central end of the cut peroneal nerve of the opposite limb. Time marked above in seconds. Signal below marking the moment of application and the duration and frequency of repetition of the faradic stimulus. In A the record is from the left vasto-crureus, the afferent spinal roots through which pass the afferent fibres of the vasto-crureus nerve being intact; in B the record is from the right vasto-crureus, the afferent spinal roots of its nerve having been severed 30 days previously. A and B are both from the same animal and experiment and were taken within five minutes of each other. The tonic after-continuance of the reflex seen in A is absent from B. At *L.r.* in A the movement of the recording surface was stopped and the extended knee was forcibly flexed and then released; the recording surface was then allowed to proceed and the horizontal line of the tracing shows that the new length passively given to vasto-crureus was now maintained ("lengthening reaction"). If the "lengthening reaction" had not been made, the tonic after-continuance of the reflex would have gone on, probably for some minutes. Time marked above in seconds.

The intensity and duration of the faradic stimulation was practically the same for both reflexes: a 100,000 ohms resistance was in the secondary circuit in both cases.

In the reproduction of the records in the figures the reduction of size has been greater in A than in B; this difference can be judged from the time line.

Fig. 3.—Crossed reflexes produced in vasto-crureus by faradic excitation of the proximal stump of the cut popliteal nerve of opposite limb. Time in seconds above : below is signal line giving duration of the faradic stimulus. The intensity of the stimulus is the same for A and for B. A is the reflex contraction of the left vasto-crureus, B that of the right ; the spinal afferent roots containing the afferent fibres of the right vasto-crureus nerve had been cut 42 days previous to decerebration and examination of the reflexes. In B the tonic after-continuance of the reflex is wanting ; on cessation of the stimulus the muscle at once relaxes completely and its slackness is indicated by the vibratory swing at its return to the base-line. In A, on cessation of the stimulus, a tonic after-contraction similar to that of the "shortening reaction" succeeds the contraction caused by the exciting stimulus itself. This was allowed to continue for 8 secs. and then the recording surface was stopped and the muscle was (*L.r.*) forcibly stretched by hand to about the length it had at first before the reflex and then released ; the recording surface was then started again and the lever traced a horizontal line at the original level, showing that the "lengthening reaction" (*L.r.*) had taken place.

In order to make the comparison fair in regard to intensity of faradic stimulus the distance between the electrodes was in both cases the same, *i.e.*, 5 mm., and the nerve-trunk was drawn between the poles ; the distance of the secondary spiral from the primary was the same for both stimulations, and a 100,000 ohms resistance was in the secondary circuit. The duration of the stimulus was managed by hand and was longer in A than in B ; that difference does not account for the difference in the duration of the two reflexes. Time marked above in seconds.

Fig. 4.—Crossed reflexes produced in vasto-crureus by mechanical excitation (quick tightening of a previously loose thread-loop) of proximal end of cut anterior tibial nerve of opposite limb. A shows the reflex response given by left vasto-crureus muscle to this excitation of right anterior tibial nerve. B shows the response given by right vasto-crureus to the similar stimulations of left anterior tibial nerve. Both reactions are from the same animal and within four minutes' interval one from the other. The spinal afferent roots containing the afferent fibres of the right vasto-crureus nerve had been severed 52 days prior to the experiment. The tonic maintenance of the reflex, which in the case of the left (A) vasto-crureus lasted with little abatement for a couple of minutes, is absent in the reflex given by the right vasto-crureus (B). Time marked above in seconds.

Fig. 5.—Crossed reflexes produced in (A) the left vasto-crureus and in (B) the right vasto-crureus by stimulation of the popliteal nerve. Stimulation by a series of weak break shocks, marked by the lowest signal. The stimuli are admitted to the nerve for the period during which the short-circuit key in secondary circuit is open, marked by the upper signal line. The afferent nerve-fibres of the right muscle (B) had been severed 80 days previously ; those of the left muscle intact. Time above in seconds.

Reciprocal Innervation of Antagonistic Muscles. Thirteenth Note.—On the Antagonism between Reflex Inhibition and Reflex Excitation.

By C. S. Sherrington, D.Sc., F.R.S.

(Received November 3,—Read December 10, 1908.)

(From the Physiology Laboratory, University of Liverpool.)

In further prosecution of observations made in Ludwig's laboratory by Schmiedeberg* and by Bowditch,† N. Baxt‡ in 1875 carried out in that laboratory a prolonged enquiry into the effect produced on the heart's frequence by combined stimulation of the inhibitory (*vagus*) and accelerator (*accelerans*) nerves going to that organ. His observations were made on large dogs; the two nerves were faradised simultaneously. In most of his experiments, stimulation of *accelerans* preceded that of *vagus* by a few seconds, to allow for the well-known longer latency of the former's reaction; the precurrent stimulation of *accelerans* alone was immediately followed by stimulation of both nerves simultaneously. Baxt found the rate of heart-beat under the combined stimulation slowed as though the vagus only and no accelerator was in operation; but, immediately following on cessation of the combined stimulation, a full accelerator effect appeared, as though no stimulation of the vagus had taken place. Baxt employed usually minimal stimulation of *vagus* and maximal of *accelerans,* for he found minimal excitation of *vagus* suffice to set aside completely, for the time being, maximal excitation of *accelerans.* In his hands, the result of the combined stimulation was vagus action, even when weakest, completely obscuring accelerans action even at strongest; but although the accelerans action showed no trace of its existence during the vagus stimulation, it appeared in full force after the vagus action had passed off. The vagus action, therefore, did not destroy it, but merely postponed its appearing. Baxt drew the conclusion that the inhibitory nerve and the excitatory nerve must act on separate points in the heart's mechanism and are not true antagonists. This view has since been endorsed by many,§ but is not that of v. Cyon, the discoverer of the *accelerator.*

Another field for examination of the same problem was chosen by

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* 'Arbeiten a. d. physiol. Anstalt z. Leipzig,' 1871.

† *Ibid.*, 1873.

‡ *Ibid.*, 1875.

§ *E.g.*, Tigerstedt, 'Physiologie d. Kreislaufs,' Leipzig, 1894; for a contrary view see S. J. Meltzer, 'Archiv. f. Physiologie,' Leipzig, 1892, p. 376, and v. Cyon, 'Nerven d. Herzen,' Berlin, 1907.

v. Frey.* He investigated the effect on the venous flow from the submaxillary gland of simultaneous stimulation of the vaso-constrictor (*cervical sympathetic*) and the vaso-dilatator (*chorda tympani*) nerves. The dilatating influence of the *chorda* proved to be completely overpowered by the constricting effect of the sympathetic during combined stimulation of the two. But on discontinuing the combined stimulation there ensued from the vein a markedly excessive blood-flow, *i.e.*, a marked chorda action ensued as an after-effect. The action of the sympathetic had, therefore, not destroyed the action of the concurrently excited chorda, it had only postponed its appearing. v. Frey, with his customary clearness, wrote†: "the antagonism between constrictor and dilatator has not as its basis a simple summing of two forces which act in opposite direction at the same point of application." The sympathetic prevails entirely for the time being as regards the surface effect, but the chorda nevertheless develops with regular course the change characteristic of its action, and does so "in some part of the excitable apparatus protected from the attack of the sympathetic: only in some other place do the actions of the two collide."‡

Some years later Heidenhain,§ discussing the acceleration of pulse-rate, which in the frog follows discontinuance of a stimulation of the vagus-trunk, attributed it to accelerans fibres commingled with the inhibitory in the vagus-trunk, and with them excited by the stimulus applied. He then went on to suggest that inhibitory actions fall into two classes which should be clearly distinguished one from the other. He argued that in one class the inhibitory process antagonises the excitatory process by decreasing or suppressing its actual occurrence. Whereas in a second class the inhibitory process, without actually lessening, or indeed altering, the excitatory process, simply prevents the latter from, for the time being, getting access to and affecting the organ of its destination, the organ which, but for the inhibition, it would reach and affect. To this second class of inhibitions he relegated the vagus action on the heart and its interference with the accelerator on that organ. As another example of this second class, he cited similarly the opposition between sympathetic and chorda on the vessels of the submaxillary gland.

This explanation of inhibition brought forward by Heidenhain as satisfying the cases which he terms inhibitions "of the second class" resembles the hypothesis earlier offered by Rosenthal.‖ Rosenthal, after making his discovery that stimulation of the afferent *laryngeus superior* brings the diaphragm to rest in the relaxed condition, offered the following explanation of this inhibition. The rhythmic action of the respiratory centre results, he argued, from the excitation which arises in that centre not having access to the motor channels directly, but having to pass a resistance. Through this resistance the continuous excitation is transformed into an intermitting and rhythmic discharge. This resistance he supposed to be increased by stimulation of the superior laryngeal nerve; whence the inhibitory action of the nerve.

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* 'Arbeiten a. d. physiol. Anstalt z. Leipzig,' 1876.

† *Ibid.*

‡ *Ibid.*

§ 'Pflüger's Archiv,' vol. 27, p. 283, 1882.

‖ 'Die Athembewegungen u. ihre Beziehungen z. N. Vagus,' Berlin, 1862.

It is interesting to find Heidenhain adopting this explanation, first advanced for a central reflex inhibition, as applicable to peripheral inhibitions (therefore of his second class), but on the other hand discarding it as little applicable to central reflex inhibitions. These latter he considered as for the most part belonging to his first class. He gives no specific instance, but he is evidently expressing the impression he has derived from his work with Bubnoff,* published the previous year, "on excitation and inhibition in Motor Cerebral Centres." In "a great number of the facts known as voluntary and reflex inhibition" it is "a matter of the weakening or suppression of central excitatory processes by willed or peripheral sensory influence."† And ten years later Meltzer,‡ from observations "on the mutual relation of the nerve-fibres in the vagus which inhibit and excite respiration," distinctly concludes that under maximal stimuli the action of one of the opposed nerves regularly hides that of the other (just as does the vagus the accelerator's action and the sympathetic the chorda dilating action), but that with submaximal stimuli "giebt es kein Ueberwiegen, sondern ein Verschmeltzen zu einer Resultante."§

Reid Hunt|| showed that, as indeed some of Baxt's own records reveal (*cf.* Meltzer), *accelerans* stimulation does express itself on beat-rate even during *vagus* excitation. O. Frank¶ obtained a similar result; he, however, upholds Baxt's conclusion as legitimate, stressing the fact that while accelerans quickens both systole and diastole, vagus slows diastole alone. Recently Bessmertny,** from experiments under Asher, also infers that these antagonists act at separate places in the cardiac mechanism. Cyon, however,

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* 'Pflüger's Archiv,' vol. 26, p. 137, 1881.

† *Ibid.*, vol. 27, p. 283, 1882.

‡ 'Archiv f. Physiologie,' Leipzig, 1892, p. 341.

§ *Ibid.*, p. 383.

|| 'Amer. Journ. of Physiol.,' 1894, vol. 2, p. 396.

¶ 'Sitzungsb. d. Ges. f. Morph. u. Physiol. in München,' 1897.

** 'Zeitschr. f. Biol.,' vol. 47, p. 400, 1905.

has always pressed an exactly opposite view.* By Asher† the question has been tested in another case by pitting depressor stimulation against asphyxial stimulation of the vasomotor centre: there also the results he concludes confirm those already given by the experiments on vagus-accelerator and on constrictor-dilatator antagonism.

Similar questions arise in regard to the reflex inhibitions expressed by skeletal muscles. Here the seat of the inhibition is central. The skeletal muscle attached by means of its motor nerve to the centre expresses by contraction the excitation of the motoneurones of that centre and by relaxation or by restraint from contraction the inhibition of those motoneurones. In the following experiments I have chosen as field for collision of the opposed influences, excitatory and inhibitory, the motoneurones of the vasto-crureus muscle. Of the various afferent arcs acting on this field I have selected two whose reflex influences are of diametrically opposed direction. Of these, one causes flexion of the knee, the other extension. The former was excited by faradisation of the central stump of the ipselateral peroneal nerve severed at the knee, the latter by similar faradisation of the contralateral popliteal nerve likewise severed at the knee. The vasto-crureus, with its nerve and blood supply and attachments intact, was isolated as described in previous papers.‡ The femur was fixed by a strong clamp close above its condyles. All nerves of the opposite leg were severed to exclude reflex movement there which might confuse the result. The animals (cat) were decerebrate, decerebration being performed under deep chloroform-ether narcosis. For faradisation two Berne coils with secondary values graduated in Kronecker units were employed. In the secondary circuit containing the electrodes there was intercalated in all cases a resistance of 100,000 ohms in order to steady the stimulation values for the nerve-trunk.

In one respect the mutual antagonism of these reflex influences acting on this preparation differs from that described as obtaining in the antagonisms mentioned previously. The difference is as follows :—When both the afferent nerves are stimulated fairly strongly the ipselateral exerts preponderant influence over the contralateral.§ This preponderance is evidenced in two ways : (α) The muscle, while contracting under the excitatory influence of the contralateral reflex, on fairly strong stimulation being applied to the ipselateral afferent nerve forthwith relaxes completely so as to show no obvious contraction (see figures in previous papers).* (β) The muscle, while lying relaxed under the inhibitory influence of the ipselateral reflex already in operation, on fairly strong stimulation being then applied to the contralateral afferent nerve still remains, in spite of that stimulus, relaxed and to all appearance devoid of any contraction (see figures in previous papers).†

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* 'Nerfs d. Cœur,' Paris, 1905, 'Nerven d. Herzen,' Berlin, 1907.

† 'Zeitschr. f. Biol.,' vol. 47, p. 87, 1905.

‡ Sherrington, 'Journ. of Physiol.,' vol. 36, p. 185.

§ Sherrington, 'Integrative Action of the Nervous System,' 1906, p. 224.

The ipselateral afferent arc is therefore the prepotent one: its action overpowers that of the contralateral arc. But, as has been shown elsewhere,‡ this prepotence is not inevitable. The influence of the contralateral afferent nerve finds clear expression if its stimulation be strong at a time when that of the ipselateral is quite weak (fig. 1). Whether the one influence or the other is preponderant depends, therefore, in the case of this preparation, on the relative intensity of the stimuli applied to the two antagonistic nerves respectively. This reversibility of the sense of the reaction seemed to me to indicate the preparation as a favourable one on which to test the nature of the antagonism. The more so as in it both the excitatory reflex and the inhibitory reflex are capable of being graded

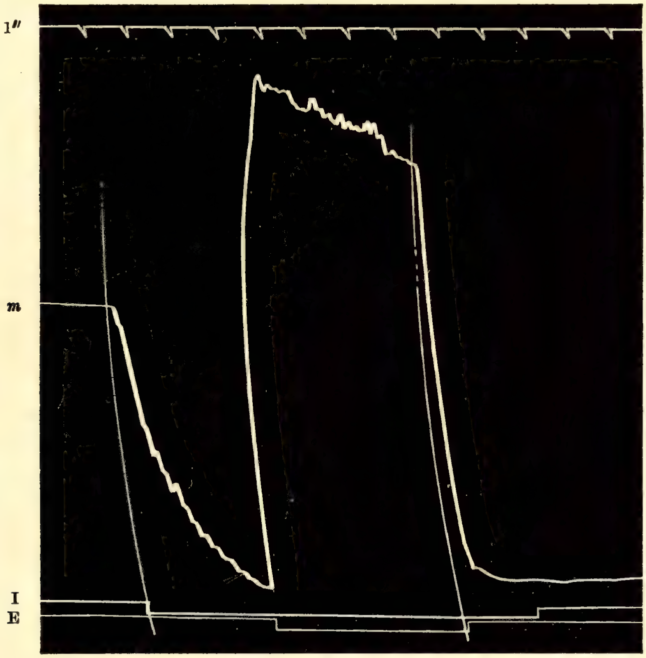

Fig. 1.

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* 'Roy. Soc. Proc.,' B, vol. 76, p. 277.

† *E.g.*, 'Brit. Assoc. Rep.,' Camb., 1904, p. 736.

‡ Sherrington, 'Integrative Action of the Nervous System,' p. 225.

in intensity with much fineness by simply grading the intensity of stimulus applied to their respective afferent nerves.*

It was sought, therefore, to find whether, when the relative intensity of the excitation of the two opposed reflexes is appropriately graded, there appears in the muscle a resultant action intermediate in degree between the relaxation which the inhibitory reflex acting by itself produces and the contraction which the excitatory reflex acting by itself produces. The accompanying reproductions of graphic records taken in typical experiments give better than any verbal description the facts observed.†

In the experiment furnishing fig. 2 the inhibitory afferent nerve (ipselateral peroneal) was faradised for the period marked by the upper signal. The secondary coil of the Berne inductorium was at 100 units on the Kronecker scale. The threshold stimulus for the inhibitory reflex lay at 40 units of the scale. It had been found that as the intensities of stimulus were increased from this threshold upward, the reflex inhibitions obviously increased in speed and persistence of effect, and also in the earlier range of the scale in depth relatively to duration of stimulus,‡ but above about 5000 units there was no clear increase further; the inhibitions became maximal. The induced currents, when the electrodes were applied to the tongue-tip, were just perceptible at 1100 units on the scale. In the myogram, relaxation of the muscle is recorded by descent of the line, contraction by ascent. The effect of the stimulation of the inhibitory afferent nerve is seen to be a fairly rapid but somewhat hesitant descent of the myogram line. This hesitancy of relaxation is, in my experience, absent from strong inhibitions, and indicates here that the inhibitory reaction, though obvious enough, was not intense. When the inhibitory reflex had been in progress about 2 seconds, stimulation of the excitatory afferent nerve (contralateral popliteal) was commenced (shown by the lower signal line), the stimulation of the inhibitory afferent nerve continuing as before. The muscle at once contracted to a certain degree. The secondary coil faradising the excitatory afferent nerve was at 500 units on the Kronecker scale. The concurrent stimulation of both nerves continued for somewhat longer than 1 second; under it the contraction of the muscle persisted at about the same grade as that which it had almost immediately attained. The stimulation of the inhibitory afferent nerve was then discontinued, that of the excitatory afferent nerve continuing as before. The muscle then at once contracted to a greater extent than

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* Sherrington, 'Quart. Journ. of Expl. Physiol.,' vol. 1, p. 98, 1908.

† The time line is above in all the figures and marks seconds. The signal lines marking the times of stimulation are below; of them the upper marks stimulation of the inhibitory afferent nerve, the lower marking stimulation of the excitatory nerve. In all the myograph lever indicates lengthening of the muscle when it descends, and shortening of the muscle when it ascends. The further description of the figures is given in the text.

‡ *Cf. ibid.*, 'Quart. Journ. of Expl. Physiol.,' *loc. cit.*

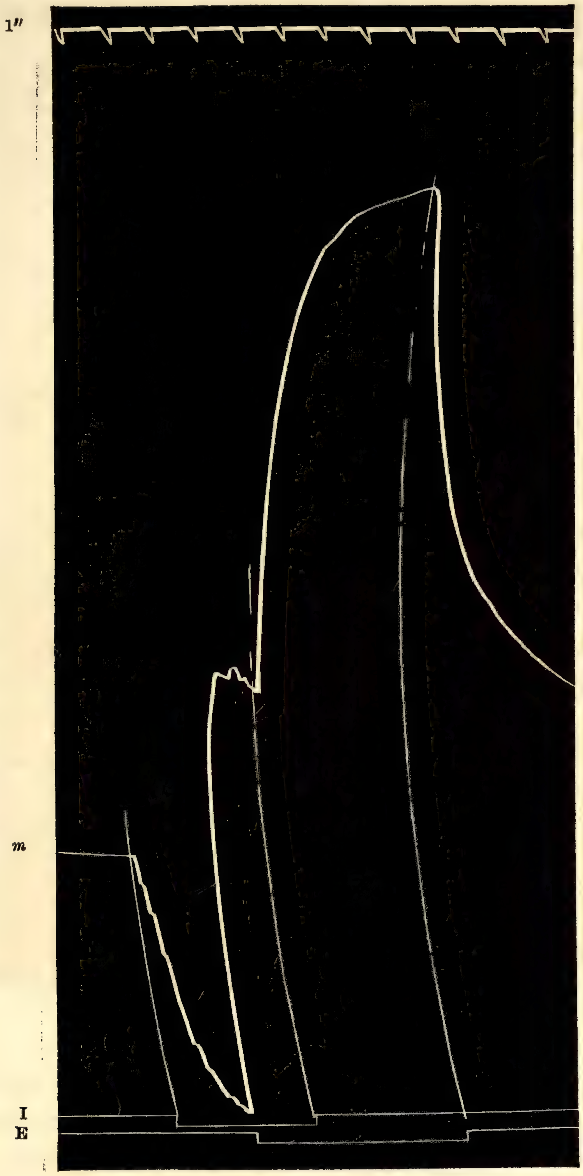

Fig. 2.

it had done under the combined stimulation of both nerves. Finally, the stimulation of the excitatory afferent nerve was discontinued. The contraction of the muscle at once diminished with gradually decreasing speed, and in about 6 seconds after cessation of the stimulus the muscle had nearly regained the length it had just prior to the observation.

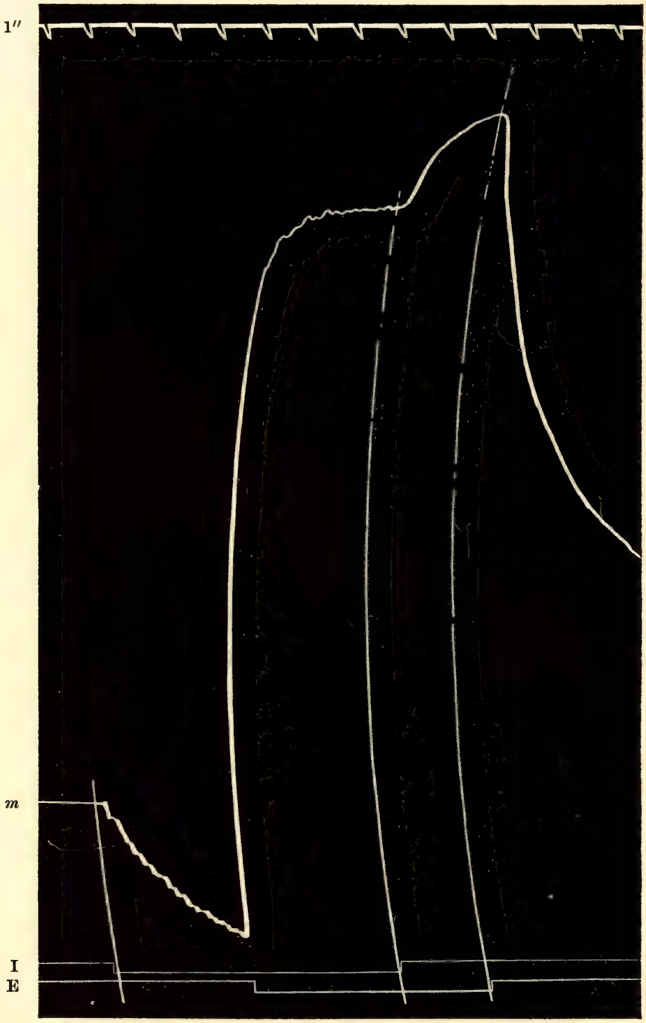

FIG. 3.

The observation of which fig. 3 reproduces the record was carried out somewhat similarly to that of fig. 2. The stimulation of the excitatory afferent nerve was of the same intensity as in fig. 2, but was continued longer. The stimulation of the inhibitory afferent nerve was, however, weaker, namely 75 Kronecker units instead of 100; it was, however, three times as prolonged. Under the simultaneous stimulation of the two nerves the muscle assumed and maintained, as the record shows, a degree of contraction greater than under the combined stimulation in the experiment furnishing fig. 2.

If the intensities of the reflex inhibition and the reflex excitation respectively be represented by the numbers on the Kronecker scale attached to the stimuli of their respective nerves, the combination Inhibition 100 + Excitation 500 (fig. 2) gives a lesser grade of contraction than the combination Inhibition 75 + Excitation 500 (fig. 3). Further, in the former combination (fig. 2) the grade of contraction is nearer to the degree of relaxed state under the inhibitory stimulus alone, and is further from the degree of contraction under the excitatory stimulus alone than in the latter (fig. 3) combination.

If figs. 2 and 3 are compared with fig. 1, they elucidate certain features of the last-named. In fig. 1, during the stimulation of the inhibitory afferent nerve (upper signal) by 80 Kronecker units, there was added stimulation of the excitatory afferent nerve (lower signal) by 400 Kronecker units. Marked contraction of the muscle resulted, and was maintained, although with gradual decrease, as long as the stimulation of the excitatory afferent nerve continued. The stimulation of the excitatory afferent nerve was then discontinued, and the muscle at once relaxed to the length it exhibited immediately before the advent of the excitatory reflex. Finally, the inhibitory stimulus was withdrawn.

From this record and from others similarly obtained the inference might be drawn that the excitatory reflex in producing the contraction entirely obliterated the action of the antagonistic inhibitory arc stimulated at the same time. Such records as figs. 2 and 3 show, however, that that was certainly not the case. On the contraction of the muscle in fig. 1, the action of the inhibitory afferent nerve is without doubt exerting influence all the time, and makes the amplitude of the contraction less than it otherwise would be. The contraction obtained is a compromise between the two antagonistic influences of excitation and inhibition simultaneously at work. The quick subsidence of the contraction on discontinuance of the stimulus of the excitatory afferent nerve in fig. 1, as compared with its slow disappearance in figs. 2 and 3, is further evidence of the continued operation of the inhibitory action in the former.

In the type of experiment exemplified by figs. 2 and 3, the period of simultaneous stimulation of the two nerves of opposed effect followed immediately on the inhibitory reflex already in operation under stimulation of the inhibitory member of the opposed pair. If the result thus given by the concurrent stimulation of the opposed pair be really of general application to their case, it should appear also when the sequence of their stimulation is reversed. The reversal was arranged, and the results it gives are exemplified by the records shown in figs. 4, B and 5, B.

In 4, B the experiment opens (lower signal line) with stimulation of the excitatory afferent nerve (contralateral popliteal) the secondary coil standing at 500 units on the Kronecker scale. The myogram shows immediate contraction of the vasto-crureus. One second later stimulation of the inhibitory afferent nerve (ipselateral peroneal, upper signal line) at 150 units of the Kronecker scale is commenced, the stimulation of the excitatory afferent nerve being continued as before. The result on the muscle is immediate decrease of the grade of contraction. The concurrent stimulation is continued for 1½ seconds, and during that period the contraction of the muscle continues to decrease more and more slowly until at end of that time the degree o

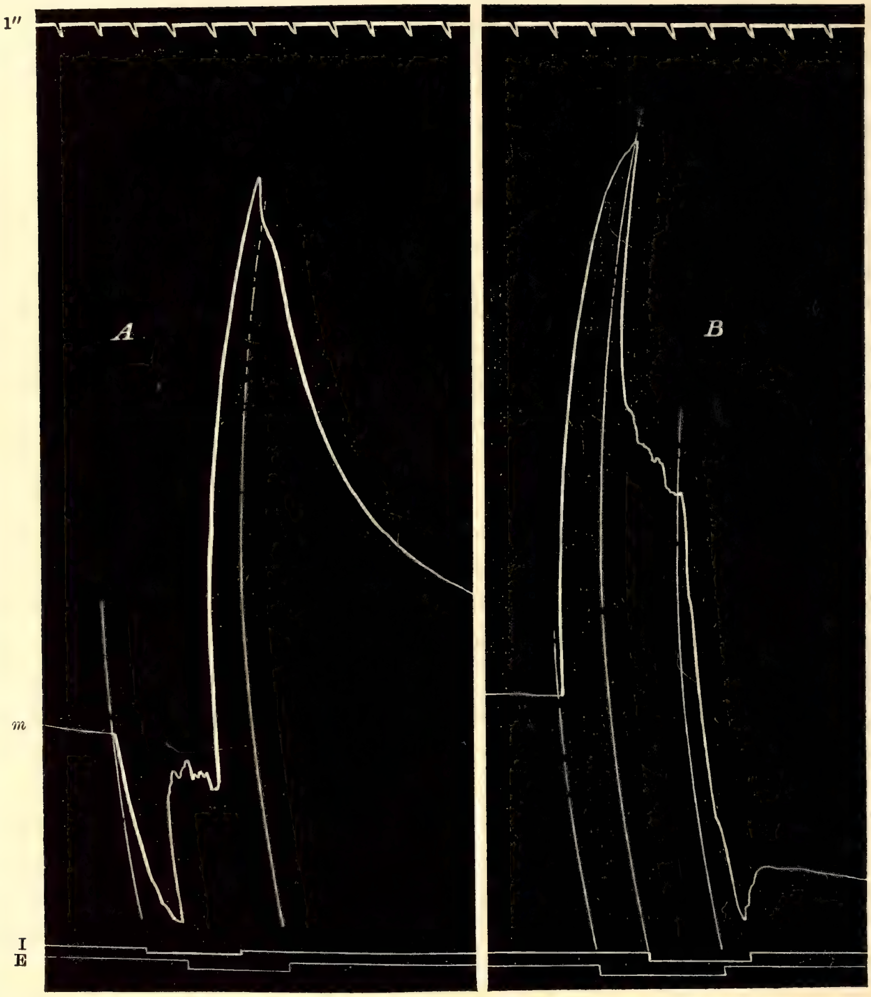

Fig. 4.

contraction has fairly settled down to a grade somewhat less than half that acquired under the stimulation of the excitatory afferent nerve alone. The stimulation of the excitatory nerve is then withdrawn: marked further relaxation of the muscle immediately follows. Finally, the stimulation of the inhibitory afferent nerve is discontinued, and this is followed by a small and relatively sluggish rebound of contraction, such as formed the subject of a previous note (successive induction).*

To assess the result of the combined stimulation in such an experiment as this it is necessary to compare the result with that obtained when, other conditions remaining unaltered, the sequence of the stimulations is made in the reverse order. This reversal was made and the record (fig. 4, A) obtained three minutes after the record, fig. 4, B. The concurrent stimulation of the antagonistic afferent nerves occurs under exactly similar circumstances as before, except that it breaks in on the reflex produced by the inhibitory component of the opposed nerves instead of by the excitatory. The height of the contraction under the combined stimulation is seen to be less in this case (fig. 4, A) than in the former (fig. 4, B). But in both cases the grade of contraction of the muscle is intermediate between that given by the excitatory afferent nerve singly and that given by the inhibitory afferent nerve singly. A further difference between the two records lies in the quicker subsidence of the contraction at the end of fig. 4, B than in fig. 4, A. This is due to the subsidence in the former being due to active inhibition. Whereas in 4, A the subsidence is due to cessation of excitation merely.

The difference just noted in the degree of contraction under the combined stimulation, according as that stimulation succeeds an inhibitory or an excitatory reflex, is greater than usually occurs. This seems referable to the brevity of the combined stimulations in these examples. That it is so is indicated by experiments such as are illustrated by the records furnishing figs. 5, A and 5, B. In fig. 5, B the experiment opens with stimulation of the excitatory afferent nerve (contralateral popliteal, lower signal line) by 500 Kronecker units. This stimulation gives immediate contraction of the muscle and the contraction has nearly reached its full height when, three seconds later, stimulation of the inhibitory afferent nerve (ipselateral peroneal, upper signal line) is commenced, stimulation of the excitatory afferent nerve continuing as before. In this experiment the stimulus applied to the inhibitory afferent nerve is very weak, namely, 50 units on the Kronecker scale, threshold value of stimulus lying at about 35 on the scale. This weak stimulation of the inhibitory nerve nevertheless immediately makes itself visible on the myogram in a slight decrease in the height of the contraction of the muscle. The

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* 'Roy. Soc. Proc.,' vols. 76 and 77, 1906.

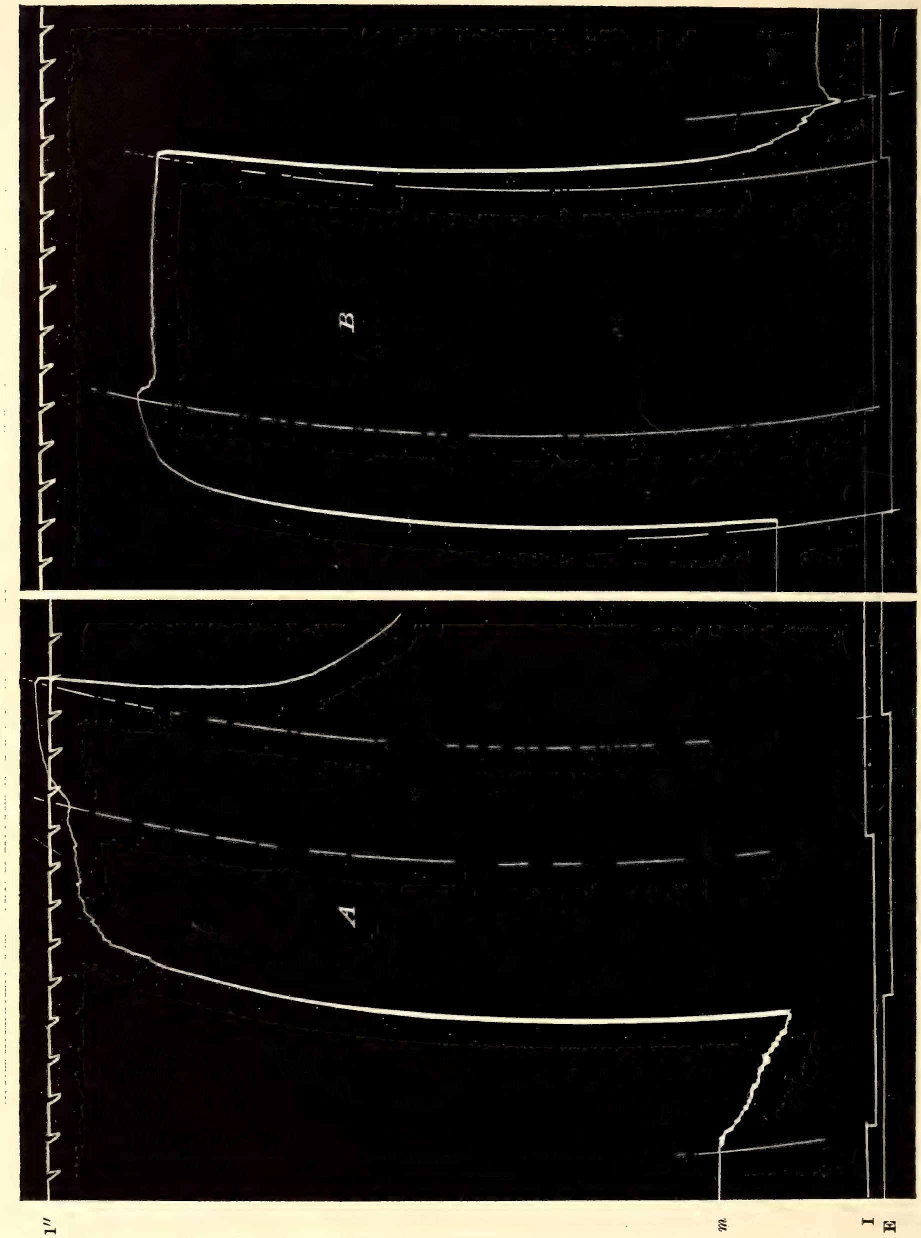

FIG. 5.

combined stimulation of the two antagonistic afferent nerves is then continued for some seven seconds and during this period there is little further alteration in the grade of contraction of the muscle. Stimulation of the excitatory afferent nerve is then discontinued, that of the inhibitory afferent nerve being continued unchanged. The muscle immediately relaxes the speed of disappearance of its contraction, documenting further the presence of inhibitory action under the stimulation, weak as it is, of the inhibitory afferent nerve. Finally, the stimulation of the inhibitory afferent nerve is withdrawn, and there then ensues a slight rebound contraction (successive induction*). With the result of the combined stimulation obtained in this case, there must be compared the result obtained a few minutes later (fig. 5, A) under similar conditions, save for reversal of the sequence of the stimulations. In fig. 5, A, the same weak stimulation of the inhibitory afferent nerve (upper signal line) opens the experiment; the slow hesitating relaxation of the muscle evidences the feeble intensity of the inhibitory influence. After this has been in progress for some four seconds, stimulation of the excitatory afferent nerve is commenced. The muscle at once contracts and in the next four seconds attains almost the summit of its contraction elicitable by the combined stimulation. That this grade of contraction is, however, less than what it would exhibit under stimulation of the excitatory afferent nerve alone is clear from what happens when the weak stimulation of the inhibitory afferent nerve is discontinued. On removal of this inhibiting stimulus the myogram line immediately ascends and a higher grade of contraction is uniformly maintained for the following three seconds, at end of which time stimulation is finally withdrawn from the excitatory afferent nerve. The muscle then relaxes, but with characteristically slower lengthening than in fig. 5, B, where the weak inhibitory stimulus was still in play at the time of withdrawal of the stimulation of the excitatory afferent nerve.

Finally a point already raised may be returned to for fuller exemplification. The records furnishing figs. 5, 3, 2, and 4 have all been chosen from a series of observations obtained in near succession and from the same preparation. The intensity of the stimulus applied to the excitatory afferent nerve remained the same throughout them all, namely, 500 units on the Kronecker scale. On the other hand, the intensity of the stimulus applied to the inhibitory afferent nerve was different in the four observations figured; in fig. 5 it was 50 units of the Kronecker scale; in fig. 3, 75 units; in fig. 2, 100 units; and in fig. 4 it was 150 units. On comparing together in the four figures 5, A, 3, 2, and 4, A the levels assumed by contraction under the combined stimulation in each case, and the relation of those levels to the depth of relaxation under inhibitory stimulus alone, and to the height of contraction under excitatory stimulus alone, it is clearly seen that the four levels form a graded series, and that the grading accords with the relative intensities of the stimuli. The length of the muscle under the combined stimulus, Excit. + Inhib., more resembles the length under

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* 'Roy. Soc. Proc.,' vols. 76 and 77, 1906.

Excit. when the Inhib. component is weak, and more resembles the length under Inhib. when the Inhib. component is strong. This result on the muscle strikingly resembles in ratio the sensual result obtained by binocularly combining dark grey and light grey simultaneously presented one to right eye the other to left.

From the observations it seems clear that the reflex effect of concurrent stimulation of excitatory afferent nerve with inhibitory afferent nerve on the vasto-crureus nerve-muscle preparation is an algebraic summation of the effects obtainable from the two nerves singly, as v. Cyon has maintained for the heart. The individual effects of the two nerves "fuse to a resultant," to use words applied by Meltzer to his results on the respiratory centre. One inference allowable from this is that in the case before us the two afferent arcs employed act in opposite direction at one and the same point of application in the excitable apparatus. They are in this sense true antagonists. The inhibition exercised by the inhibitory member of the pair comes therefore under Heidenhain's Class I of inhibitory reactions. As to the common locus of operation, the point of collision of the antagonistic influences, it seems permissible to suppose either that it lies at a synapse, in which case the opposed influences may be thought of as altering oppositely the permeability of the synaptic membrane, or that it lies in the substance of the "central" portion of a neurone, probably of the motoneurone itself, meaning by "central" that part of the neurone which lies in the reflex centre. In either case the condition of the material of the common locus seems altered in two diametrically opposite ways by the two antagonistic afferent arcs. The net change which results there when the two arcs are stimulated concurrently is an algebraic sum of the *plus* and *minus* effects producible separately by stimulating singly the two antagonistic nerves.

[Reference was made above, p. 568, to observations on antagonism which took the vasomotor centre as test-object. On this subject a very clear statement is given by Bayliss.* "The results of exciting simultaneously the depressor nerve and a pressor nerve, such as the central end of the anterior crural, depend entirely on the relative strengths of the stimuli: whichever nerve is under the stronger excitation shows its own effect on the blood-pressure and when the excitation of this one is stopped the effect of the other manifests itself." "There appears to be a perfect antagonism between the pressor and depressor actions on the centre." In the same work, Prof. Bayliss describes also the result of opposing depressor action against asphyxial stimulation, the latter when weak lessening the effect of the former, when strong annulling it.—January, 1909.]

* 'Journ. of Physiology,' vol. 14, p. 318, 1892.

PROCEEDINGS OF THE ROYAL SOCIETY.
SERIES B, VOL. 80, Appendix, 1908.

OBITUARY NOTICES

OF

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energetic advocacy that Cambridge to-day possesses a flourishing agricultural school, and it was a great source of delight to him when the University determined to follow his lead and provide for the teaching of agriculture on a scientific and practical basis.

Zoology shared with botany his active interest; it was due largely to his co-operation with Ray Lankester, and his active support, that the Marine Biological Research Laboratory at Plymouth came into existence; an instrument of research which, with other kindred establishments, has proved of the utmost value to the progress of zoology.

His power of grasping and presenting to others a mass of complicated detail was remarkable. As senior Secretary he had for many years to bring the business to be dealt with before the Council of the Royal Society, and through his skilful presentations of the facts, the Meetings ended with but few arrears. He held his own views tenaciously, but accepted an adverse vote with good humour. He was keenly alive to the desirability of keeping the Council in close touch with the Society, and it was largely owing to him that the existing custom of the presentation of an annual report by the Council to the Fellows was introduced.

Foster was a delightful companion, full of humour, with an irresistible bubbling over kind of laugh, and a twinkle in his eye which was most infectious. He was excellent as an after-dinner speaker, and was usually expected to speak; frequently, some unexpected humorous but never ill-natured allusion to some distinguished person at the dinner, would cause roars of laughter; almost as frequently the laugh would be directed against himself.

Up to the very last he was at work, up to the last he retained his gift of humorous speaking, and on the very day on which he died (January 28, 1907), he had made an excellent speech in London at the Meeting of the British Science Guild.

This last public appearance was a fitting end to a great career. There have been many greater scientific men than Foster. It is hardly too much to say that no man ever devoted himself more whole-heartedly to science, and that if science can be served by strengthening the influence and promoting the spread of the scientific spirit, few have done it better service.

W. H. G.

CHARLES STEWART, 1840—1907.

Charles Stewart was born at Plymouth in 1840 and died in London on September 27, 1907. After obtaining the qualification of M.R.C.S. in 1862, he returned to Plymouth, where his father and grandfather had been medical practitioners, and for a short time followed the same profession. In 1866, however, he left Plymouth for London, and from that time devoted himself entirely to Zoological and Physiological teaching and investigation. For nearly twenty years he spent his chief energies in lecturing and teaching at St. Thomas' Hospital and at Bedford College, besides, we believe, doing much incidental lecturing in the provinces. At St. Thomas' he held successively the lectureship in Comparative Anatomy, to which he was appointed in 1871, and a joint lectureship in Physiology (with Dr. John Harley) which he obtained ten years later. The necessity of earning an income set him upon this path, which was, however, pursued with skill and success.

There is no doubt that Prof. Stewart was one of the best lecturers in London. This opinion was indeed fully recognised by his nomination to deliver many Friday evening lectures at the Royal Institution and his appointment to the Fullerian Professorship of Physiology in that Institution from 1894 to 1897. Prof. Stewart's abilities as a lecturer were not exhibited in the way of oratorical display and the use of gesture. His merit was a smooth and even flow of language, simple in character and at times not merely unconventional but even familiar. Combined with this was an obvious interest in, and mastery of, the subject with which he was dealing, which carried conviction and aroused sympathy. He possessed also in a high degree that faculty so necessary to a lecturer upon biological subjects, the power to illustrate his statements upon the blackboard. The present writer heard him lecture upon fishes in the Zoological Society's House at Hanover Square on an occasion of afternoon lectures inaugurated by the Society, but since discontinued.

Discarding altogether the use of lantern or wall diagrams, Prof. Stewart depicted with rapidity and accuracy upon the blackboard coloured representations of certain tropical fishes in which even the iridescent hues of those creatures were skilfully suggested with simply a few coloured chalks.

Prof. Stewart's life work was, however, accomplished during his tenure of the office of Curator of the Museum of the Royal College of Surgeons. Though mainly devoted to teaching before his selection to the headship of that incomparable Museum, he had held the minor office of Curator of the

Museum at St. Thomas' Hospital. The performance of the duties of that post indicated a talent for museum work hardly inferior to his powers as lecturer and teacher and one which continually improved by use during his long occupancy of the Curatorship of the College of Surgeons Museum.

The duties, however, of that important office were, at the time of Prof. Stewart's appointment, as they had been in times past, not merely those of a museum superintendent. The Conservator of the Museum discharged also the duties of Hunterian Professor and was expected to deliver courses of lectures. Until the last few years of his tenure of the Conservatorship, Stewart gave annually a course of six or nine lectures upon various topics, though connected, as was natural, with the extensive collections under his control. The first series, delivered in 1885,* were upon the "Structure and Life History of Hydrozoa"; subsequently he lectured upon "Auditory Organs" (in 1886 and 1887), on "Phosphorescent Organs" (in 1890), on "Alternation of Generations" (in 1899), on the "Integumental System" (in 1889, and again in 1896), and on a variety of other subjects, the lectures generally, or at any rate frequently, laying special stress upon recent acquisitions to the Museum.

The wide outlook upon biology implied by these lectures and by Stewart's full appreciation of John Hunter's idea of a museum is perhaps responsible for the comparatively small amount of detailed zoological discovery published by him. Endeavouring to give the most liberal interpretation to the idea of "Physiological Series," Prof. Stewart devoted himself to illustrating copiously from the vegetable as well as from the animal kingdom the facts upon which anatomical and physiological generalisations were based. This left but little time for the writing of memoirs upon new anatomical facts; we find, therefore, that but little share was taken by Stewart during these years in the affairs of learned societies, whether as a contributor or as an office holder. For four years, however (1890—1894), he held the important position of President of the Linnean Society, but his contributions to the publications of that Society had been made in earlier years.

The zoological work accomplished by Stewart, though not large in amount, and chiefly published during the "'seventies," contained some important new matter. His main claim to distinction as a zoologist rests upon his work upon the Echinodermata. He discovered in the genus *Cidaris* certain organs, subsequently called after him "Stewart's organs," believed to be of a respiratory nature, but whose functions are not yet certain. Other papers upon the Echinodermata are: "On the Spicules of the regular Echinoidea," 'Trans. Linn. Soc.,' 1866; "On the minute Structure of certain Hard Parts in the Genus *Cidaris*," 'Quart. Journ. Micr. Sci.,' 1871; "On some Structural Feature of *Parasalenia*, etc.," 'Micr. Journ.,' 1880, and one or two others. His most recent memoir published in the 'Journal of the Linnæan Society' for 1907 is upon "The Membranous Labyrinth of certain Sharks," and Prof. Stewart also contributed to the 'Quarterly Journal of Microscopical Science' some notes upon the teeth of *Ornithorhynchus* and to the Zoological Society some details in the anatomy of the poisonous lizard *Heloderma*, especially relating to the glands which secrete the poison. In addition to these papers upon Echinodermata and vertebrated animals, Prof. Stewart published a few memoirs upon Corals and other Invertebrates. In all he wrote about twenty papers.

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* The writer is indebted to Mr. R. H. Burne for this and other information.

In reviewing Stewart's contributions to scientific literature, the "Catalogues" of the Royal College of Surgeons are by no means to be omitted. These recent Catalogues differ in their form and in many other particulars from the older Catalogues published during the tenure of the Conservatorship of the Hunterian Museum by Sir Richard Owen. The volumes are of smaller size, are illustrated by illustrations in the text (with an occasional Plate), and are most of them at any rate almost of the nature of treatises on the subjects with which they deal.

Stewart was not merely the editor of the Catalogue, in itself a large piece of scientific work, but wrote a great part of it, especially of the first volume.

Although hardly able himself to devote much time to the preparation of scientific memoirs, Stewart was exceedingly generous to others and anxious to assist them with the abundant material at his command. Of this the present writer can speak from his own experience. Stewart's enthusiasm for zoological anatomy was, in fact, perfectly disinterested. A total absence of even the capacity for sordid scheming, and a disposition which was, as a contemporary justly remarked of him, "unassuming to a fault," rendered Charles Stewart an ideal official and endeared him to those who were privileged with his friendship.

F. E. B.

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The Library and Offices will be closed for cleaning from August 24 to September 5.

All books borrowed from Library are returnable by August I, and no books can be borrowed during August.

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